DIGESTATES FROM FOOD WASTE AND LIGNOCELLULOSIC MATERIALS: EFFECTS ON PLANT GROWTH

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Abstract

Anaerobic digestion is a process that converts organic matter into two useful products: biogas, which can be used for energy, and digestate, which has potential as a fertilizer and soil amendment. The majority of research on digestates focuses on their fertilizer value. However, there is a lack of information about other effects they may have on plant growth, both positive and negative. Understanding the effects of digestates on plant growth is essential to optimizing their use in agriculture, and helping to close the loop of energy production. A series of experiments were conducted to assess the potential presence and activity of phytohormone-like compounds in a food waste digestate (FWD) and a lignocellulosic digestate (LCD). In preliminary laboratory experiments, bioassays suggested that there may be hormone-like activity. Further research would be needed to determine the active compounds responsible for these effects. In addition, a greenhouse experiment was conducted to test the effects of digestates in comparison with a synthetic nutrient solution made to mimic their mineral and nutrient content. Both the FWD and the LCD increased plant growth significantly more than their synthetic nutrient equivalents and showed a quadratic-like response to increasing rates of digestates in early growth of *Brassica juncea*. A second greenhouse study evaluated the effects of digestates, mineral fertilizers and combinations of the two on plant biomass, root growth and nutrient use of *Brassica juncea* plants. Combinations of LCD and mineral fertilizer performed as well or slightly better than the fertilizer control for most parameters, including aboveground biomass and root length. These same combinations had significantly higher nitrogen use efficiency than the fertilizer control. There were inhibitory effects observed with pure LCD treatments, likely due to the high EC of the media from digestate application. Based on this research, LCD could partially replace mineral fertilizers for *Brassica juncea* at up to 50% of the target nitrogen rate and may lead to increased plant growth beyond mineral fertilizers. FWD could replace up to 100% of the target nitrogen application without causing significant negative effects on plant growth. Further research is needed both to verify these findings under field conditions and with different species of plants to determine optimum rates of application.

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Chapter 1.

Introduction

The demand for locally produced food continues to rise both nationally and in the state of Hawaii (The Kohala Center, 2014). However, the Hawaiian Islands face unique challenges in increasing local production to meet this demand. Currently, farmers import the majority of fertilizers, fuel and seeds for local food production, leaving the island state particularly vulnerable to price fluctuations, scarcity and natural disasters. Hawaii's fertilizer prices are higher than the continental U.S. largely because of shipping costs. Increasing availability and accessibility of local fertilizers and other products that improve plant growth will offset some of the higher costs of agriculture in Hawaii. This in turn will contribute to making agriculture in the state both less vulnerable to external pressures and more competitive with U.S. mainland agriculture. Ultimately, this improves livelihoods for local farmers while helping to satisfy the increasing demand for affordable, locally produced food in Hawaii.

In addition, there is an excess of waste, especially organic waste, that could be recycled into useable products. In Hawaii, waste is an especially critical issue because of the limited land mass for disposal. On Oahu, the majority of the more than 2.2 million tons of waste produced annually is burned, producing toxic ash and air as well as greenhouse gases (Kokua Foundation). Of that, more than 35% is organic waste (Cascadia Consulting Group, 2018). Worldwide, an estimated 1/3 of food produced for human consumption is discarded, totaling about 1.3 billion metric tons of food waste alone per year (Gustavsson et al. 2011). On Oahu alone, over 150,000 tons of food waste were produced in 2017, making up more than 20% of overall waste (Cascadia Consulting Group, 2018).

Lastly, there is a recognized dependence on fossil fuels for energy. This dependence on fossil energy has created energy insecurity in much of the world, along with significant environmental costs, including production of greenhouse gases and degradation of air quality. Anaerobic digestion (AD) is a promising technology with the capability of turning a variety of organic materials, including organic waste, into two potentially useful products: biogas for energy and the effluent, hereafter referred to as "digestate," as a potential fertilizer and soil amendment (Khanal, 2008).

Anaerobic digestion (AD) is the series of processes by which organic materials are degraded in the absence of oxygen to produce methane. The process can be described in 4 major steps. The hydrolysis phase involves the initial breakdown by bacteria of insoluble polymers into simpler molecules for further degradation. The acidogenic phase converts sugars and amino acids into even smaller molecules including carbon dioxide, hydrogen, ammonia and organic acids. The acetogenic phase involves conversion of these molecules into acetic acid, ammonia, hydrogen and carbon dioxide. Lastly, the methanogenic phase converts these into methane and carbon dioxide. The primary product is biogas, largely composed of methane, which can be used to generate electricity or refined into natural gas and fuels. The effluent that remains, referred to as biogas slurry, biogas residue, and hereafter termed “digestate” can be utilized as a nutrient-rich fertilizer and soil amendment. Various feedstocks are used in AD, ranging from waste products (i.e. manures, agricultural wastes, food waste) to dedicated energy crops, including various grasses and grains. The use of waste products in AD provides additional benefits including net energy production rather than consumption and mitigation of the need for costly waste disposal, while also producing a potentially high-value soil amendment.

While much of the research on anaerobic digestion has focused on its potential as an energy source, there is a need for more research on the fertilizing and plant-growth promoting capabilities of the digestates produced as a co-product of this process. There are still conflicting results and unknowns with respect to digestate’s potential as a fertilizer. Better understanding the effects of digestate will help inform and optimize digestate’s use in agriculture (Nkoa, 2014; Möller and Müller, 2012; Teglia et al, 2011).

1.1 Literature Review

1.1.1 Fertilizer and Amendment Value of Digestates

A soil amendment can be defined as a material that improves or maintains the physical, chemical and/or biological properties of soil (Nkoa, 2014). Organic matter content is the main indicator, as higher organic matter suggests a higher amendment value. A fertilizer, on the other hand, promotes plant nutrition directly through the addition of plant nutrients with the goal to increase yields. Much of the research on the use of digestate in agriculture has focused on its fertilizer and nutrient value, but it can also be considered an amendment due to its organic matter content and subsequent beneficial effects on soil biochemical parameters (Garg et al., 2005).

Digestates generally have a high ammonium content as a percent of total nitrogen (Abubaker et al., 2012, Möller and Müller, 2012; Wentzl and Joergensen, 2016). Ammonium nitrogen (NH_4-N) is a mineral form of nitrogen that can be used directly by plants or readily converted into plant available nitrate, thus leading to increased plant growth. Digestates have potential as a valuable source of short-term fertilizer nitrogen, particularly in organic cultivation where there is a lack of quick-release fertilizers (Furukawa and Hasegawa, 2006; Möller and Müller, 2012). Digestates in organic vegetable production have shown favorable results in spinach, tomatoes and lettuce (Furukawa and Hasegawa, 2006; Fang-Bo Yu et al., 2010; Liu et al., 2011).

Digestates are generally considered to increase the availability of plant nutrients in comparison with raw materials (Möller and Müller, 2012). Multiple studies have found increased nitrogen (N) availability and use efficiency with digestates as compared with undigested materials (Möller et al., 2008; Gunnarsson et al., 2011). In general, the composition of digestates and their fertilizer value is largely dependent on the feedstock used and can vary significantly between digestates (Teglia et al., 2011; Alburquerque et al., 2012). Digestates made from feedstocks with a higher N content generally have a higher ammonium content, varying from 45-80% of the total N (Möller and Muller, 2012). Although most research discusses fertilizer value of digestate with respect to nitrogen, there is a general consensus in the literature that it is also a valuable source of phosphorus (P), and depending on the feedstock, potassium (K) as well (Tambone et al., 2010; Ronga et al., 2019).

1.1.2 Effects on Plant Growth and Yields

Digestates are generally considered to have a fertilizer value between raw manures and commercially available fertilizers (Nkoa, 2014). However, previous research shows varying and sometimes contradictory results concerning the effects of digestates on crop yield. The type of control used in the experiment must be taken into account, as there are three main controls used in studies on digestates: unamended, raw materials and mineral fertilizer.

Svensson et al. (2004) and Wentzel and Joergensen (2016) found that plants grown with digestates had growth similar to or better than an unfertilized control. Multiple studies have found increased plant growth with digestate than with undigested materials (Chantigny et al., 2007; Möller et al., 2008; Wentzel and Joergensen, 2016). Perhaps most agronomically relevant are those studies assessing the performance of digestate in comparison with commercial fertilizers. Several studies have found similar or improved growth with digestates over mineral fertilizers (Chantigny et al., 2008; Ahmad and Jabeen, 2009; Gunnarsson et al., 2010; Haraldsen et al., 2011). In addition, one study investigated dry matter production over a long-term field study and found that growth of *Lolium multiflorum* was lower with digestate at 40 days and higher than mineral fertilizers past 136 days (Gunnarsson et al., 2010).

While much of the research finds that increases in plant yield are largely related to the NH_4-N content of digestates, there are suggestions that the benefits from digestates may go beyond simply fertilizer value. Wentzel and Joergensen (2016) found that increases in above-ground yield were not linearly related to applied N. In addition, they saw that benefits from digestate continued beyond the first cut of ryegrass, even though there were no additional inputs. Various research suggests that there may be other factors, both positively and negatively affecting plant growth, beyond their nutrient value alone. These factors vary from effects on soil biochemical parameters to the impact of added organic matter in digestates to the possible interplay of various phytohormones and bioactive compounds found in digestates. More research is needed to understand what the effects of specific digestates are on the growth of specific plant species. In addition, a better understanding of possible mechanisms for digestate effects beyond their nutrient value alone will help optimize their use in agriculture.

1.1.3 Hormone-like Effects of Digestates

There is some research suggesting that digestates contain bioactive compounds that can promote plant growth and increase tolerance to stress (Kostenberg and Marchaim, 1993; Liu et al., 2009; Yu et al., 2016; Li et al., 2016). Various studies have observed the effects of digestates on hormone-driven processes such as germination and early root growth stimulation/inhibition (Möller and Müller, 2012). One such study found that soaking seeds in digestate increased germination and root length of wheat seedlings (Gurung 1997). Another found that spraying digestate increased grain number and thousand-grain weight in wheat (Garg et al., 2005).

Alburquerque et al (2012) found increased germination, initial root growth and higher seedling biomass in both cress and lettuce with the addition of low concentrations of digestate in comparison with the control. However, these studies did not further investigate the compounds that could be causing the observed effects. These results suggest possible plant growth regulating properties or phytohormone-like effects of some digestates. Salminen et al (2001) et al found an inhibition of root growth and germination due to digestate treatment that was correlated with various organic acids including palmitic acid and fatty acids. The inhibitory effect varied based on the type of digestate used, and no single common factor or compound was found to be responsible for the effects seen in different plants.

Previous research has found elevated amounts of indoleacetic acid (IAA) in digesates. IAA is an active form of the plant growth regulating hormone auxin that may be produced by microbes during the digestion process (Kostenberg et al., 1995; Li et al, 2016; Scaglia et al., 2015). At low concentrations, auxin has been shown to promote root growth, but at higher concentrations, it can inhibit root growth (Mo et al., 2004). Kostenberg et al. (1995) reported IAA in digestate from instant coffee waste and attributed increased rooting with ornamental plants to the presence of this hormone. Ertani et al (2013) found an auxin-like effect of the humic-like fraction of digestates on maize plant growth and suggested that digestates should be considered as plant-growth promoters. Scaglia et al (2015, 2017) found that IAA present in the dissolved organic matter fraction of digestate from pig manure was the compound likely causing the greatest hormone-like effects. However, it’s important to consider that these last two studies were both working with fractionated and/or purified forms of digestate, which may not always be a practical approach for on-farm use. Overall, there is lack of quantitative research on the hormone-like effects of unfractionated digestates, as they would likely be applied on farms.

1.1.4 Potentially Negative Attributes of Digestates

Digestates have been touted for their beneficial effects on plant growth. This is largely due to their fertilizer and/or soil amendment capabilities, as well as their potential bioactivity and hormone-like effects. However, digestate use in agriculture in not risk-free, and the level of risk with digestate application depends on multiple factors, including the feedstock utilized. There is some concern regarding heavy metal content, however research has shown that the content of heavy metals in digestate is generally below established thresholds (Nkoa, 2014). In addition, the use of certain feedstocks, including animal manures and sewage sludge, introduces the possibility of elevated levels of pathogenic organisms including *Salmonella* spp. and *Escheria coli*. However, the digestion process has been shown to reduce the number of pathogenic species (Alburquerque et al., 2012; Nkoa, 2014; Qi et al., 2018). Lastly, there is research suggesting that digestates may have phytotoxic effects related to their excessive NH_4-N content, the presence of certain organic acids, and/or their high salinity (Salminen et al., 2001; Abdullahi et al., 2008; Alburquerque et al., 2012). The occurrence of phytotoxic effects can also vary based on the feedstock used, digestate composition, concentration of digestate applied and plant species being grown. More research is needed to understand how to best mitigate potential negative effects of digestates while gaining optimum benefits for crop growth and reducing environmental impacts.

1.2 Research Goal and Objectives

The goal of this thesis research is to better understand how digestates affect plant growth in an effort to optimize their use in agriculture. In order to achieve this goal, this study consists of the following objectives:

1) Quantify the presence and activity of hormone-like compounds in digestates made from different feedstocks.
2) Assess and compare the capabilities of digestates to improve plant growth beyond their nutrient value.
3) Evaluate the effects of combining digestates and mineral fertilizers on root growth and nutrient use efficiency of plants.

The thesis is presented in three chapters. The first chapter focuses on efforts to understand the presence and activity of hormone-like compounds in digestates through a variety of techniques. The second addresses the question of whether digestates have effects beyond their nutrient value, and compares the performance of two different digestates in the early growth stages of *Brassica juncea* (var. Hirayama). The third chapter accounts for the possible negative effects of digestates applied at full strength. It quantifies the effects of digestates alone and in and combination with fertilizers on both aboveground and belowground plant growth with a particular emphasis on root growth parameters and nutrient use.

Chapter 2.

Exploratory Research on the Composition and Bioactivity of Digestates.

2.1 Introduction

Much of the research on the use of digestate in agriculture focuses on its fertilizer and nutrient value. Digestates generally have a high ammonium content as a percent of total nitrogen (Abubaker et al, 2012, Möller and Müller, 2012). Ammonium is a plant-available form of nitrogen which can be used directly by plants or readily converted into plant available nitrate, thus leading to increased plant growth. The addition of ammonium N can also lead to priming effects in the soil, stimulating microbial activity and nutrient cycling (Bernal and Kirchmann, 1992, Gunnarsson et al, 2010). Digestates have potential to be a valuable source of short-term fertilizer nitrogen, especially in organic cultivation where there is a lack of quick-release fertilizers (Möller and Müller, 2012). Digestates in organic vegetable production have shown favorable results in tomatoes and lettuce (Yu et al, 2010, Liu et al, 2011). They've been shown in other studies to produce similar or increased yields when compared with mineral fertilizers (Abubaker et al, 2012; Haraldson et al., 2011; Svensson et al., 2004).

Two main research gaps exist with respect to digestate use as a soil amendment. First, and most importantly for this research, there is not a clear and complete understanding of the mechanisms involved in improved plant growth with digestates. While the fertilizer value of digestates has been well-studied, other mechanisms for increased plant growth and yield are less clear. Second, there is a lack of research on the characteristics and growth-promoting capabilities of digestates made from feedstocks available in Hawaii. Better understanding the mechanisms that affect plant growth with the addition of digestates made from locally available feedstocks will help inform future research and development of this potentially high-value soil amendment.

Previous research on digestates suggests an additional mechanism for improved plant growth that goes beyond its fertilizer value. Two such studies found higher crop yields and/or biomass from digestates than from mineral fertilizers with the same amount of mineral N applied (Abubaker et al, 2012; Tampio et al, 2016). No clear explanation or mechanism has been confirmed for these results. However, several studies have investigated the presence of phytohormones in digestates. These suggest the possible role of phytohormones and hormone-like compounds in improving plant growth with digestate use (Li et al, 2016, Möller and Müller, 2012).

Phytohormones are signal molecules in plants that regulate cellular processes, growth, and defense. They are active at very low concentrations and are naturally produced by both plants and microorganisms. Of particular interest in digestates are the plant hormones that promote or inhibit plant growth. Plant hormones involved in growth regulation include auxins, cytokinins, brassinosteroids, jasmonic acid, salicylic acid and ethylene. Of particular interest is auxin, the most comprehensively studied and cited hormone involved in plant growth. In addition to these plant hormones, many other organic molecules have been linked to plant growth and regulation in similar ways as auxin, including organic acids and amino acids (Colla et al, 2014; Scaglia et al, 2015; Scaglia et al, 2017).

Different feedstocks have been shown to produce digestates with different fertilizer and plant growth-promoting effects (Tambone et al, 2010; Abubaker et al, 2012). Tambone et al (2013) saw differences in nutrient profiles as well as differences in the chemical make-up of the carbon compounds in digestates made with different feedstocks. Differences in nutrient content are significant with respect to the fertilizer value of digestates. In addition, differences in the composition of feedstocks could be influencing plant growth beyond the fertilizer value of digestates. However, the relative contribution of nutrients and other plant growth-promoting effects of digestates remains largely unknown. Understanding how feedstocks affect the role of digestates in improving plant growth may be critical in optimizing their use in agriculture.

The exploratory research presented in this chapter describes efforts to better understand the composition of digestates, beyond their nutrient value, both in order to identify compounds that contribute to plant growth and to quantify phytohormone-like effects of digestates. We investigated a food waste digestate rich in nitrogen (N) and a grass-based digestate low in N. Also included in this chapter are some of the specific shortcomings and difficulties with the aforementioned efforts, as well as possible future directions to achieve a more complete understanding of digestate activity.

2.2 Digestate Production and Characterization

2.2.1 Food Waste Digestate (FWD) Production

The food waste digestate was produced via semi-batch anaerobic digestion at the University of Hawaii at Manoa. Food waste was collected from Hale Aloha Café over a one-week period to account for the effects of slight menu changes on food waste composition. The resulting mix of food waste was blended until homogenized. The inoculum used was collected from the final digestion tank at East Hawaii Wastewater Treatment Plant in Hawaii Kai. Three samples each of both the food waste and inoculum were weighed and dried in an oven at 105˚C for 48 hours and weighed to calculate total solids (TS). They were placed back in the oven at 550˚C for an additional 48 hours and weighed to calculate volatile solids (VS).

Food waste and inoculum were mixed at a 2:1 ratio on a volatile solids basis at an organic loading rate of 15 g of VS/L. Both inoculum and food waste were added to a 2 L glass bottle for a total of 6 replicates and deionized water was added to make up the remainder of the 1.5 L working volume. Three control batches were made up of only inoculum and deionized water to account for gas production by the inoculum itself. Bottles were sealed and connected to gas collection bags. All bottles were placed in a shaking incubator under mesophilic conditions at 37˚C for 65 days. Gas volume was measured with a Ritter drum-type gas meter (Bochum, Germany) and gas composition was measured via gas chromatography.

In order to increase the proportion of food waste without overloading the system, additional food waste was added twice during the digestion process. Additional food waste was added at 50% of the initial loading rate once the system reached a plateau of gas production, once at day 30 and again at day 44. The effects of this food waste addition can be seen in the spikes immediately following food waste addition in the gas production curve in Figure 2.1.

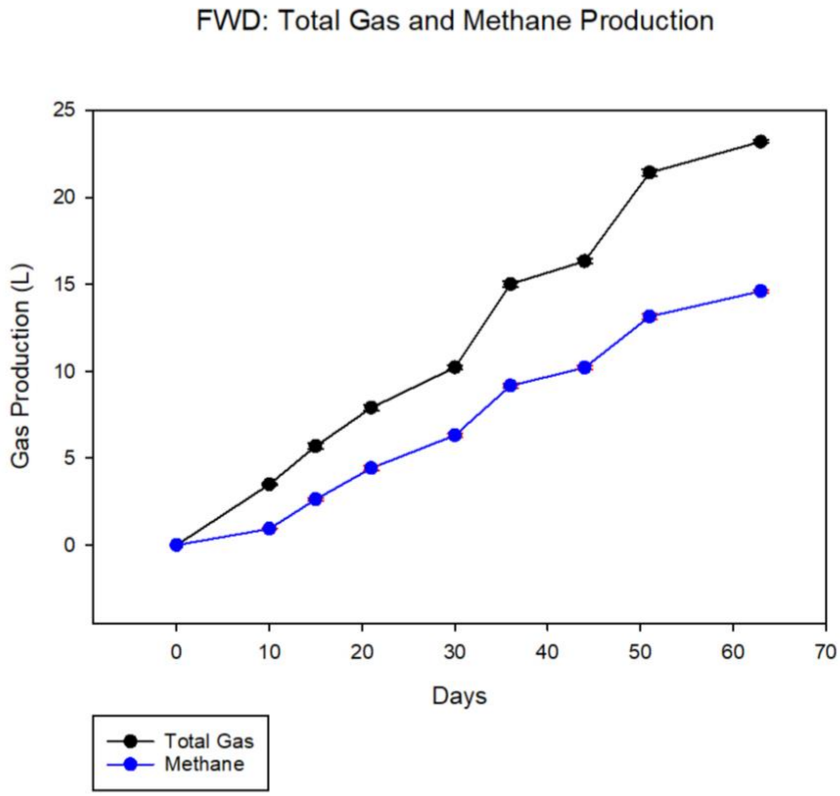


Figure 2.1: Gas production curve for food waste digestion. Error bars are SE of the mean. Note: additional food waste added at days 30 and 44.

2.2.2 Lignocellulosic Digestate (LCD) Production

The lignocellulosic digestate (LCD) was produced via semi-continuous digestion in the Khanal laboratory. The primary feedstock consisted of dried and ground Napier grass (*Pennisetum purpureum*) grown at the Waimanalo Research Station, University of Hawaii at Manoa. Six-liter horizontal flow reactors were operated under mesophilic conditions (35 +/- 2˚C). Reactors were fed with biomass at an organic loading rate of 4g VS/L.d for semi-continuous digestion (Phuttaro et al., 2019). Specific methane yield was between 0.168-0.243 Nm^3 (kg VS_{added})$^{-1}$ depending on plant part used (Surendra et al, 2018). Digestate was collected every 2 days for a period of 3 weeks and combined for use in this research.

2.2.3 Characterizing Digestates

Digestate samples were sent to DairyOne Analytical Laboratory (Ithaca, NY) for chemical analysis. The characteristics of both digestates are summarized in Table 2.1 and reflect differences in both physical and chemical parameters. Overall, the FWD was enriched in essential plant nutrients, especially ammonium nitrogen as well as phosphorus, calcium and iron, while the LCD contained a higher concentration of potassium. Both digestates had a high electrical conductivity (EC) as well as high amounts of both sodium and chloride, attributes that must be taken into account when considering their value as fertilizers due to their potential negative effects on plant growth.

Table 2.1: Physical and chemical properties of the digestates.

Parameter	***Lignocellulosic Digestate***	***Food Waste Digestate***
Total Solids	2.87%	1.21%
pH	8.4	8.6
Electrical Conductivity (mS/cm)	8.34	10.99
Total Nitrogen (mg kg^{-1} ww)	710	1790
Ammonium Nitrogen (mg kg^{-1} ww)	270	1150
Organic Nitrogen (mg kg^{-1} ww)	440	640
Nitrates (mg kg^{-1} ww)	0	0
Phosphorus (mg kg^{-1} ww)	120	460
Potassium (mg kg^{-1} ww)	920	380
Calcium (mg kg^{-1} ww)	90	250
Magnesium (mg kg^{-1} ww)	110	130
Sulfur (mg kg^{-1} ww)	70	410
Sodium (mg kg^{-1} ww)	910	930
Chloride (mg kg^{-1} ww)	2500	1300
Iron (mg kg^{-1} ww)	29	496
Zinc (mg kg^{-1} ww)	0.9	13.1
Copper (mg kg^{-1} ww)	1	3.7
Manganese (mg kg^{-1} ww)	1	1

2.3 Auxin-Specific Bioassay

2.3.1 Introduction

Previous research suggests the presence and activity of phytohormones in digestates. Various studies have observed the effects of digestates on hormone-driven processes such as germination and early root growth (Möller and Müller, 2012). A study by Alburquerque et al (2012) found increased germination, initial root growth and higher seedling biomass in both cress and lettuce with the addition of low concentrations of digestate (1% by volume) in comparison with the control. However, their study did not further investigate the compounds that could be causing the observed effects. These results suggest possible plant growth regulating properties or phytohormone-like effects of some digestates.

Most of the research on phytohormones in digestates has focused on auxin and auxin-like compounds. Kostenberg et al (1995) found auxin in the form of indole-3-acetic acid (IAA) to be present in anaerobically digested instant coffee waste. Furthermore, they postulated that this IAA was likely responsible for the promotional effect of the same digestate on the rooting of ornamental plants compared with undigested wastes (Kostenberg and Marchaim, 1993). Scaglia et al (2015) examined the dissolved organic matter fraction (DOM) of digestates for hormone-like activity as seen in a root inhibition bioassay. Based on this study, they suggested that auxin (IAA) was primarily responsible for the hormone-like activity in digestates from pig slurry. They further suggested that this auxin was a product of the decomposition of tryptophan (Ramirez and Garraway, 1981). They attributed the auxin effect seen with other digestates to hydroxyphenylacetic acid, another active auxin molecule that results from the breakdown of lignin, as well as other aromatic and fatty acids (Tanaka et al, 1990). Scaglia's analyses showed the auxin-active molecules and fatty acids to be the most important contributor to the auxin effect (2017). Li et al (2016) conducted a study to evaluate the presence of phytohormones at various stages of anaerobic digestion and under different storage conditions. They found auxin to be the most likely hormone in digestate responsible for observed plant growth regulating effects. Furthermore, they suggested that the auxin content of digestates was enhanced by microbial synthesis from the amino acid tryptophan during digestion (Ramirez and Garraway, 1981). Salminen et al (2001) found inhibition of root growth and germination due to digestate treatment that was correlated with various organic acids including palmitic acid and fatty acids. The

inhibitory effect varied based on the type of digestate used, and no single common factor or compound was found to be responsible for the effects seen in different plants.
Multiple studies have found an auxin-like effect from various digestates, but did not actually deduce what compounds were responsible for the effect (Kostenberg, 1995; Ertani et al, 2013). Scaglia et al (2017) isolated auxins and auxin-like compounds from a variety of digestates. They tested different fractions of the dissolved organic matter from digestates for an auxin-induced growth response using a bioassay-based procedure. However, there is no known bioassay that has tested specifically and definitively for the auxin effect in plants treated with digestates.

Chen et al (2014) have worked extensively with an *Arabidopsis thaliana* mutant plant in studying the transport and production of auxin in plants. This particular mutant, *yucQ*, has a mutation on 5 of the genes involved in the primary auxin production pathway (*yuc3/5/7/8/9). yucQ* plants display a phenotype in which root growth upon germination is extremely stunted in comparison with the wild type plant. Previous studies with this plant reported average root growth of 9 +/- 3 mm for mutant plants while wild type plants had an average root growth of 20 +/-4 mm after 5 days. Because this plant responds specifically to auxin, it is an ideal candidate for studying the presence and effects of auxins in digestates. Most important for the purposes of this study, normal root growth can be rescued by exogenous application of auxin (Chen et al, 2014). Our experiment was designed to look specifically at the activity of auxin in digestate using this auxin-deficient mutant.

2.3.2 Methods

An auxin-specific bioassay was designed using the *Arabidopsis thaliana* mutant plant (*yucQ*) to quantitatively determine auxin activity in different digestates. *yucQ* seeds were acquired from Dr. Yunde Zhao and Dr. Julin Maloof from the University of California at San Diego and Davis, respectively. Plants from these seeds were grown at the Pope Greenhouse Facility to ensure an adequate number of seeds for the experiment. Col-0 *A. thaliana* seeds were used as the wild type since that is the background genotype for the *yucQ* mutant.

The experiment was set up as a completely randomized design in a growth chamber. The two factors of interest are seed type (wild type (WT) versus *yucQ (YQ)*) and treatments consisted of a deionized water control, a nutrient control, a standard auxin control at 5 nM, food waste digestate (FWD) at 1% and 10% by volume, lignocellulosic digestate (LCD) at 1% and 10% by volume. Digestate treatments were split into cold-sterilized treatments and unfiltered treatments to test for possible microbial interactions.

Seeds were surface sterilized and stratified in 0.1% agarose solution at 4°C for 3 days to break dormancy. All media, with the exception of the water control, contained ¼ strength Hoagland solution and sucrose. Using this medium ensures that seedlings receive sufficient nutrients and that the effects seen are due to differences in auxin compounds rather than nutrients in the digestates. The negative control treatment contained 2 ml of sterile deionized water (DIW). Seeds were grown in a growth chamber set to 22°C with a 16 hour photoperiod. Five seeds each of WT of YQ were affixed to each plate with agarose.

2.3.3 Results, Challenges and Future Directions

Figure 2.2 below shows the WT seeds in columns 1-3 and the mutant seeds in columns 4-6 of the deionized water control plate both for this experiment. According to the study by Chen et al (2014), YQ seeds should exhibit a strongly stunted root phenotype in the DIW control treatment in comparison with the WT seeds. However, in this experiment, the *yucQ* mutant seeds exhibited extremely varied phenotypes under control conditions and did not show stunted growth due to auxin deficiency as would be expected. In fact, some mutant seeds appeared to show a stimulated growth pattern in water, which is contrary to expectations.

Figure 2.2: Observed root phenotype: Arabidopsis seedlings after 7 days in this experiment.

The variability of the seeds can be seen clearly in the histograms of Figure 2.3. WT seeds show a normal distribution with a small standard deviation, while the YQ seeds show a very wide distribution without a single distinct peak.

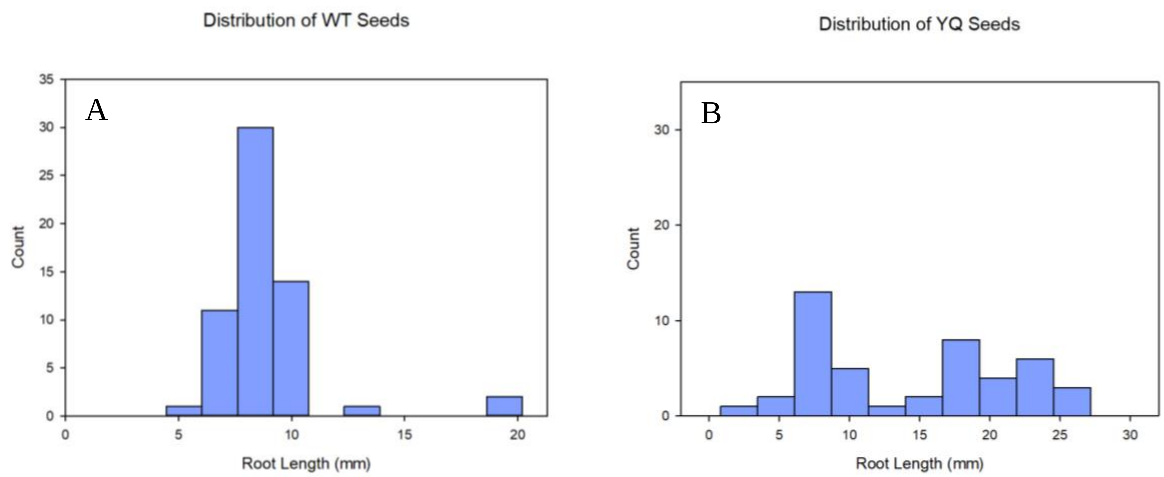


Figure 2.3: Distribution of root lengths in (A) wild type (WT) seeds vs. (B) yucQ *mutant (YQ) seeds.*

The combined variation and lack of typical response of the mutant seeds suggest that the seeds may have been contaminated or that the mutant was unstable due to the yuc7 mutation likely being inherently unstable (Zhao, personal communication, February 13, 2019). Unfortunately, this rendered any results from the experiment unusable. If clean and stable seed stock became available, this experiment could provide significant insight into the importance of auxin-like activity in not only digestates, but any biofertilizer suspected to produce auxin-like effects.

2.4 Assay-Based Fractionation to Identify Bioactive Compounds in Digestates

2.4.1 Assay-Based Fractionation

We used assay-based fractionation techniques to identify compounds in digestate that may be contributing to hormone-like effects on root growth. The approach involved a 48-hour bioassay to assess the stimulatory and/or inhibitory effects of digestates on early root growth with cucumber as the test plant. The bioassay was based on previous work with both digestates and other proposed biostimulants used to test for hormone-like effects that inhibit or stimulate early root growth (Scaglia et al, 2017, Pizzeghello et al, 2006; Wang et al, 2001). Figure 2.4 below provides a schematic overview of the steps in the process.

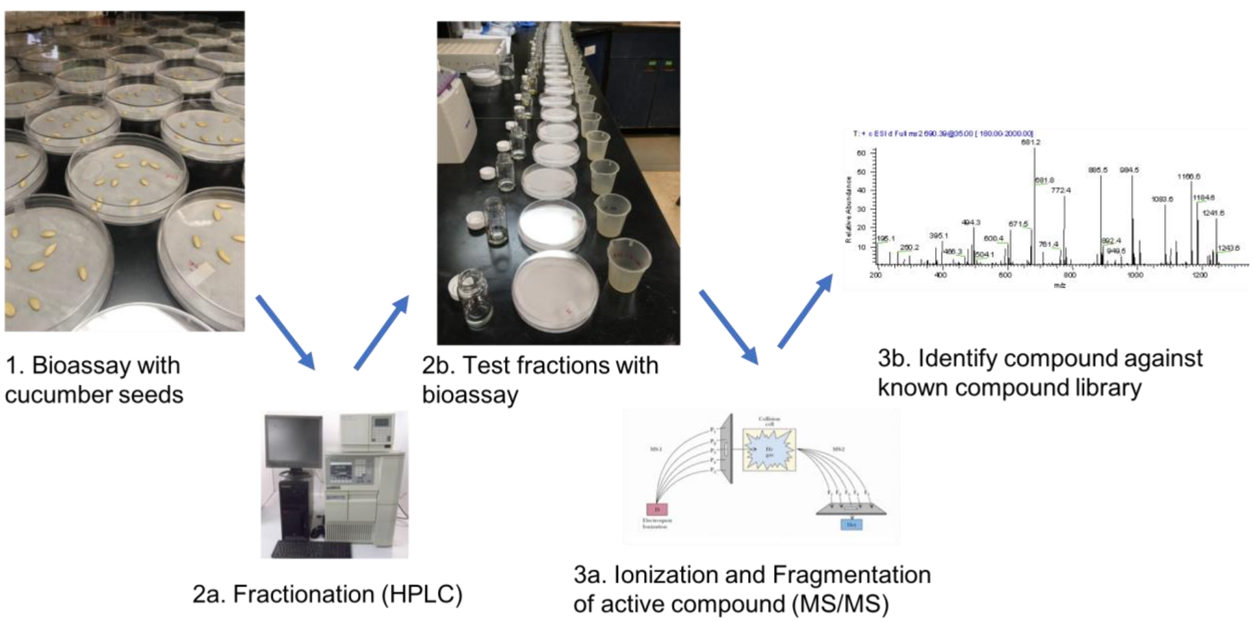


Figure 2.4: Graphical overview of assay-based fractionation.

Briefly, filter paper was wetted with 2 mL of varying concentrations of digestate in petri plates. Treatments included 0.1%, 1%, 5%, 10%, 20%, 50% and 100% (volume fraction) of each digestate, along with a 0% deionized water control. Digestates were diluted with deionized water to achieve desired concentrations. Ten cucumber seeds were placed in each petri dish and each treatment was replicated 3 times for a total of 30 seeds per treatment. Treatments were arranged in a completely randomized design and seeds were kept in darkness under room temperature and high humidity conditions for 48 hours.

After 48 hours, root lengths were measured from the tip of the root to the point where the radicle originated. Root lengths below 5mm were considered non-germinated seed (Wang et al, 2001). Root lengths were normalized using the control sample to report a length index according to equation 2.1:

$$LI = \frac{\text{root length of treated seeds}}{\text{root length of control seeds}} \qquad [2.1]$$

where LI is the length index. A stimulatory effect was considered present if the length index was greater than one and there was a significant difference between the treated seeds and the control (deionized water). An inhibitory effect was considered present if the length index was less than one and there was a significant difference between the treated seeds and the control (Scaglia et al, 2017).

2.4.2 Bioassay Results

There was a significant difference in root length due to treatment (Fig 2.5; $p<0.001$). Both digestates showed significant inhibitory effects on root growth at 50% and 100% concentration with $LI<1$ and significantly different root length than the deionized water control.

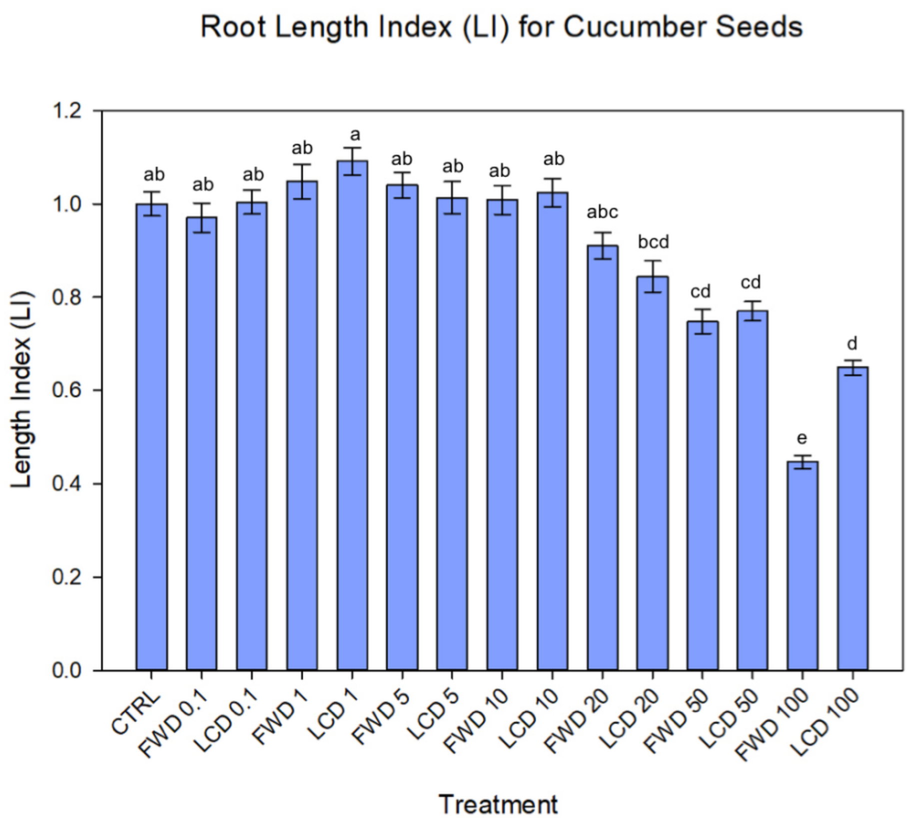


Figure 2.5. Root length index with different concentrations of digestates. Error bars are SE of the mean. Treatments that do not share a letter are significantly different (Tukey's HSD, α=0.05).

There was a slight, though not statistically significant, stimulatory effect (LI>1) on early root growth with low concentrations of both FWD and LCD. This is similar to the results reported by Alburquerque et al (2012) who saw an increase in lettuce and cress germination and seedling biomass in a similar bioassay with 0.1% and 1% digestate solutions and suggested possible biostimulatory effects of digestates at low concentrations. There was a significant inhibitory effect (LI<1) of digestates at higher concentrations of both LCD and FWD at digestate concentrations greater than 20%. At very low concentrations, auxin can have stimulatory effects, while at higher concentrations, it can inhibit root growth (Pitts et al, 1998). Scaglia et al (2017) utilized a similar bioassay to test for auxin-like activity of the dissolved organic matter fraction of digestates and suggested that a significant inhibitory effect with LI <1 indicated auxin-like activity. *Based on these results, both digestates would be suspected to have auxin-like effects.* FWD was hypothesized to have a greater hormone-like effect than LCD due to its higher protein content. A greater amount of amino acids in the FWD would lead to a higher amount of auxin and auxin-like compounds in this digestate due to the breakdown of tryptophan and other amino acids during the digestion process (Ramirez and Garraway, 1982; Scaglia et al., 2017). However, the only significant difference between FWD and LCD was at the 100% digestate level, and the greater inhibition observed was likely due to other factors such as the higher EC and salinity of the FWD.

Both digestates had high EC as well as high concentrations of both Na^+ and Cl^-. Previous research has shown a strong negative correlation between EC and soluble salts in digestate and both germination and early root growth (Alburquerque et al, 2012). This, rather than hormone-like effects, may help explain the observed inhibitory effects at higher concentrations of both digestates. However, the slight though non-significant stimulatory effects at lower concentrations may still be explained by hormone-like activity.

2.4.3 Initial Assay-Based Fractionation of LCD

We used the bioassay above to test each of the fractions from the initial fractionation of LCD with High Performance Liquid Chromatography (HPLC). First, the LCD was freeze-dried and reconstituted in concentrated form. Next, this concentrated LCD was fractionated on a Waters 2695 HPLC system (Milford, MA) based on its affinity to the HPLC column. The resultant fractions were tested using the same 48-hour bioassay with cucumber seeds to assess for stimulatory or inhibitory effects.

The bioassay results from the fractions were compared against a deionized water control using Dunnett's test (Fig 2.6; $p<0.001$). Fraction 6 had an LI significantly higher than 1, suggesting stimulatory effects. Fraction 15 had an LI significantly lower than 1, suggesting inhibitory effects on root growth indicative of possible auxin-like effects.

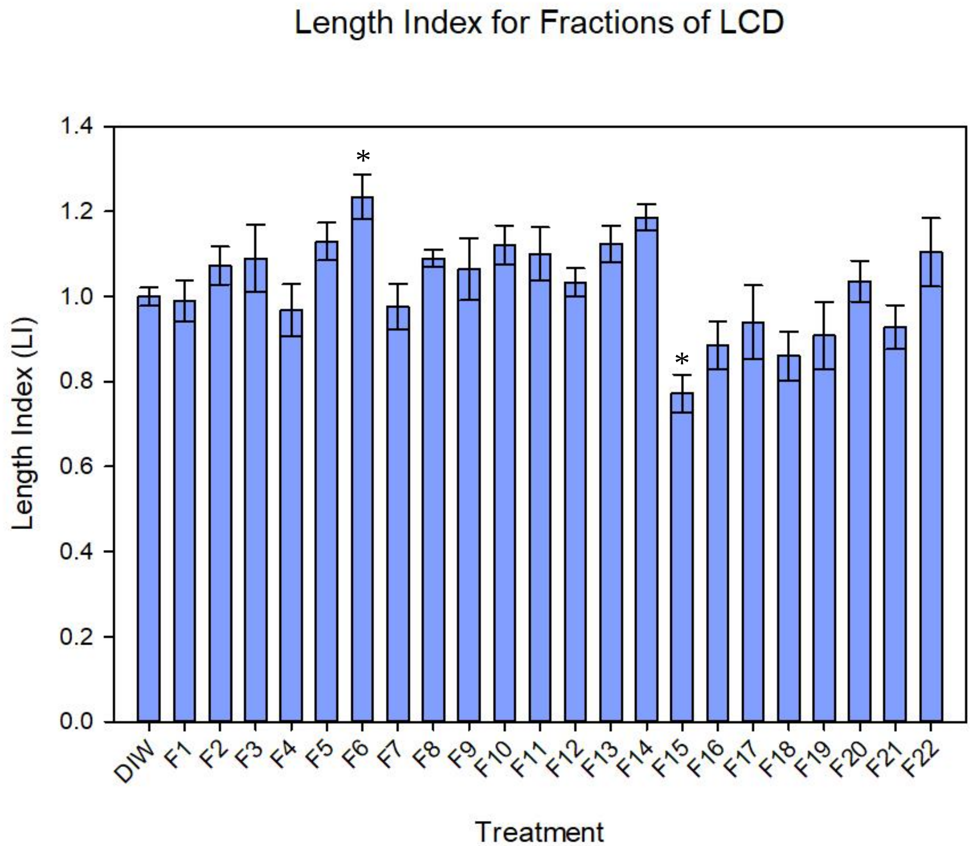


*Figure 2.6. Root length index of fractionated LCD. Error bars are SE of the mean. *indicates that a fraction was significantly different from the control (Dunnett's test, α=0.05)*

Further fractionation of the active fractions would be the next step towards identification of active compounds within digestate. In this case, fractionation would continue until active fraction(s) showed a single and distinguishable peak on a chromatogram. This peak would then be identified using mass spectrometry and comparison with a known library of phytoactive compounds. However, that work is beyond the scope of this project and I chose to focus more directly on the effects of digestates on plant growth, morphology and function.

2.5 Conclusion

A significant inhibitory effect of digestates was seen in a cucumber bioassay, indicating possible auxin-like effects of both FWD and LCD. Initial fractionation of LCD produced fractions with both stimulatory and inhibitory effects, indicative of hormone-like activity. The research presented in this chapter suggests that further investigation of the hormone-like effects of digestate should be conducted. Considering the differences in chemical composition of the two digestates used in this research, we would also expect differing compositions in carbon compounds found in each that could be contributing to bioactivity of these digestates (Tambone et al, 2013). More specifically, further research should include a comparison of different digestates made from different feedstocks to assess the possible varying effects of feedstock on both presence and activity of bioactive compounds in the digestates. Initial fractionation of LCD showed potential inhibitory and stimulatory fractions, both of which may be contributing to the growth effects observed with its application to seeds at different concentrations. Beyond a general characterization of bioactive compounds in digestate, there is particular interest in understanding the relative role of auxin and auxin-like compounds in digestates, because of their ability to regulate and stimulate plant growth. If clean seed stock were available for the *yucQ* mutant used in the auxin-specific bioassay, that bioassay could serve as a valuable and specific tool for screening potential biostimulants for auxin activity.

Chapter 3.

Beyond Nutrient Value: Plant Growth with Digestates vs. Equivalent Mineral Solutions

3.1 Introduction

Digestates have largely been studied with respect to their fertilizer value. Several studies have shown similar or even increased plant growth with digestates compared to mineral fertilizers (Abubaker et al., 2012; Haraldsen et al, 2011; Gunnarsson et al, 2010). Various studies have found bioactive substances including phytohormones and other plant-growth promoting compounds (Liu et al, 2009; Yu et al, 2010; Scaglia et al, 2015). Many researchers attribute the beneficial effects of digestates over mineral fertilizers to the presence of these phytohormones and other phytostimulatory compounds.

However, the majority of studies investigating plant growth with digestates compare plant growth with digestates versus a fertilizer composed of only nitrogen, phosphorus and potassium (NPK). This does provide valuable agronomic comparisons to assess the usefulness of digestates as a supplement or replacement for mineral fertilizers. Specifically, it does not provide an adequate control for assessing which effects are attributable directly to nutrients in digestates and which may be attributable to other factors.

In order to first verify whether there was indeed a beneficial effect of digestates on plant growth beyond their fertilizer value alone, a more robust control was necessary that more closely matched the nutrient profile of digestates. The present research consisted of the following primary objectives:

1) Evaluate the effect of two different digestates, a lignocellulosic digestate (LCD) and food waste digestate (FWD), and their respective synthetic mineral equivalents during early growth stages of *Brassica juncea.*
2) Evaluate the biomass production of *Brassica juncea* grown with digestates at varying rates of NH_4-N and a commercially available mineral fertilizer in order to assess the fertilizer potential of the two digestates.

With respect to the first objective, we hypothesized that both digestates would improve plant growth beyond their mineral equivalents, and that FWD would perform better than LCD due to bioactive compounds that stimulate plant growth. For the second objective, we hypothesized that digestates would improve plant growth beyond the commercially available fertilizer at the same rate of NH_4-N application for the same reasons. Furthermore, we hypothesized that less digestate would be required to achieve the same level of plant growth due to other factors in digestates that improve growth beyond nutrients alone.

3.2 Materials and Methods

3.2.1 Experimental Growing Conditions

The experiment was conducted in the Pope Greenhouse facility at the University of Hawaii at Manoa. Half-gallon pots were filled with 110 g oven-dried equivalent of Sunshine Mix #1 media (Sun Gro Horticulture, Agawam, MA). We determined field capacity by filling pots with air dried soil and placing them in a basin of water. Pots were left in the basin for 48 hours until the media was fully saturated by capillary action and then allowed to drain, covered, for an additional 48 hours until dripping ceased. At that point, I weighed the pots again, and the final weight minus the weight of the pot was considered field capacity. This was used to calculate the amount of water needed to reach field capacity for a given quantity of air dried media.

Brassica juncea (var. Hirayama) seeds were sown in flats to ensure even germination. After one week of growth, seedlings were transplanted into ½ gallon nursery pots filled with Sunshine Mix #1. Pots were arranged in a randomized complete block design on a single greenhouse bench with 4 replicates per treatment. Pots were then weighed every 1-2 days to determine the amount of water needed throughout the growing season. We watered plants by hand to maintain pots at field water capacity throughout the experimental period.

3.2.2 Digestates and Mineral Solutions

The digestates used in this experiment were produced in the Khanal laboratory at the University of Hawaii (Chapter 2). The lignocellulosic digestate (LCD) was produced from Napier grass (*Pennisetum purpureum*, a grass grown specifically for the production of biogas) via semi-continuous anaerobic digestion. The foodwaste digestate (FWD) was produced from foodwaste collected from the UH Manoa cafeteria and produced via semi-batch anaerobic digestion. The chemical properties of the digestates are shown in Table 3.1 alongside their mineral equivalents.

Table 3.1: Characteristics of the digestates and equivalent mineral solutions.

Parameter	***LCD***	***NE (LCD)***	***FWD***	***NE (FWD)***
Total Solids	2.87%	0%	1.21%	0%
Electrical Conductivity (mS/cm)	8.34	n/a	10.99	n/a
pH	8.4	n/a	8.6	n/a
Total Nitrogen (mg kg^{-1} ww)	710	270	1790	1150
Ammonium Nitrogen (mg kg^{-1} ww)	270	270	1150	1150
Organic Nitrogen (mg kg^{-1} ww)	440	0	640	0
Nitrates (mg kg^{-1} ww)	0	0	0	0
Phosphorus (mg kg^{-1} ww)	120	120	460	460
Potassium (mg kg^{-1} ww)	920	920	380	380
Calcium (mg kg^{-1} ww)	90	90	250	250
Magnesium (mg kg^{-1} ww)	110	110	130	
Sulfur (mg kg^{-1} ww)	70	5	410	635
Sodium (mg kg^{-1} ww)	910	910	930	
Chloride (mg kg^{-1} ww)	2500	2960	1300	2600
Iron (mg kg^{-1} ww)	29	1	496	1
Zinc (mg kg^{-1} ww)	0.9	1	13.1	1
Copper (mg kg^{-1} ww)	1	1	3.7	1
Manganese (mg kg^{-1} ww)	1	1	1	1

Synthetic mineral solutions were made using laboratory reagents to match each digestate chemical profile as closely as possible. Mineral solutions matched their respective digestate for major plant nutrients including ammonium nitrogen, phosphorus, potassium, calcium and magnesium, as well as potentially harmful sodium. Some differences occurred due to available reagents for making the solutions. Sulfur content was slightly higher in the FWD nutrient equivalent, and slightly lower in the LCD nutrient equivalent. Chloride content in both mineral solutions slightly exceeded that in the digestates. Micronutrients including iron, zinc, copper and manganese were added at a rate of 1 ppm to both nutrient solutions.

3.2.3 Treatments

Treatments were determined based on the amount of mineral nitrogen (N) applied in the digestates. Fertilization was based on nitrogen recommendations of 56 kg ha^{-1} (50 lbs $acre^{-1}$)for leafy greens grown in organic soils (Hochmuth et al, 1994). This amount was doubled to ensure adequate nutrition in the constrained volume of media for the treatments receiving 100% of the recommended fertilizer N. Therefore, the 100% nutrient rate for both digestates and nutrient solution was established at 112 kg ha^{-1} N or 518 mg/kg N based on a depth of 15 cm and a bulk density of 0.144 g cm^{-3}. Ammonium N was considered as the only nitrogen source in digestates. No nitrates were measured in either digestate and shorter growing periods have been shown to produce little to no mineralization of organic N in previous studies with digestates (Gunnarsson et al, 2010). Control treatments included water (0% fertilizer) and MiracleGro at 100% of the fertilizer rate. Digestate and nutrient solution treatments included 1%, 10%, 50% and 100% of the recommended fertilizer N for a total of 18 treatments.

Digestate and nutrient treatments were split evenly into 4 treatments and applied on a weekly basis via fertigation. The specified volume of treatment liquid was mixed with water to a total volume of 100 mL and added to the base of plants. The first application was done at transplanting and subsequent treatments were applied every week for a total of 4 applications. Plants were harvested 4 weeks after transplanting.

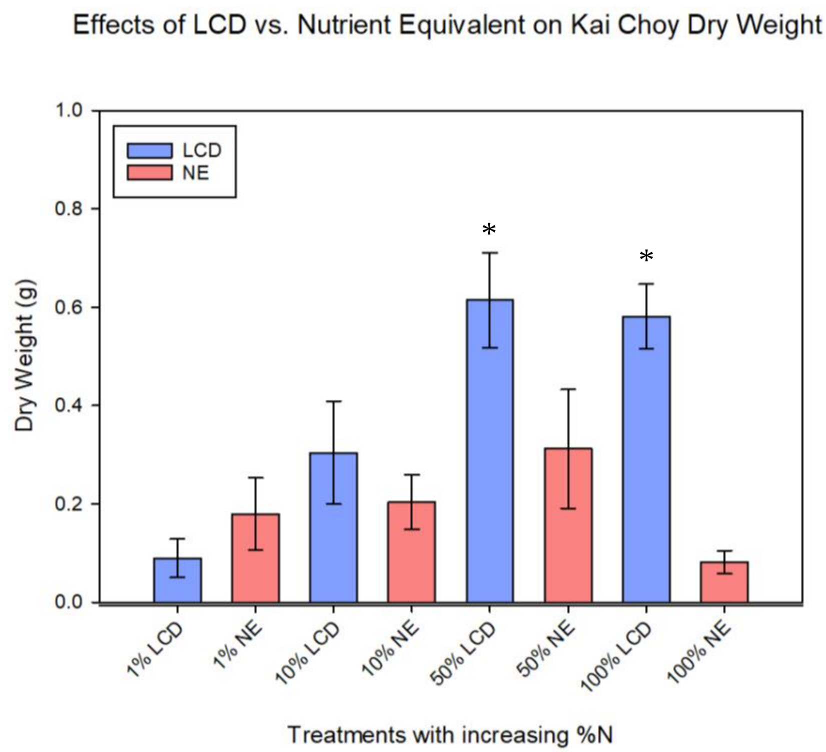
3.2.4 Analyses and Statistics

After four weeks, whole plants were destructively harvested. We cleaned roots of any remaining media under a steady stream of water over a fine mesh sieve to recover any remaining pieces of root. Clean plants were placed into weighed and labeled paper bags and dried in an oven at 70˚C for 48 hours until weight remained constant. Dry weight data was analyzed for each digestate using regression to compare the effects of nutrient source (digestate vs. nutrient solution) and rate of fertilization at 95% probability. Regression analysis was done on each of the digestates and nutrient solutions with increasing rates. We used analysis of variance (ANOVA) at 95% probability to assess significant differences among all treatments at both 50% and 100% nutrient rates. Mean values were separated using Tukey’s HSD. All statistical analyses were performed in MiniTab 18 (State College, PA).

3.2 Results

3.2.1 Plant Biomass with Digestates vs. Nutrient Solutions

Figure 3.1 shows a comparison in dry biomass between each of the digestates and their nutrient equivalents (NE). In both digestate and nutrient equivalent comparisons, the nutrient equivalent solution did not improve plant biomass with increasing amounts of NH_4-N applied (R-sq(adj) = 0.004), while the digestates showed increased plant biomass at both 50% and 100% of NH_4-N applied. All treatments produced low biomass in the 1% and 10% rates of NH_4-N applied, indicative of nitrogen deficiency. Plant biomass appeared to plateau with LCD application at 50% and did not increase at 100% of NH_4-N added. In the FWD and nutrient equivalent comparison, a similar trend was observed up to 10% NH_4-N, where neither FWD treated plants nor nutrient equivalent treated plants produced much biomass, again indicative of nitrogen deficiency. Similarly, at 50% and 100% NH_4-N, plants treated with FWD increased in biomass while those treated with nutrient equivalent did not. While plants treated with LCD appeared to not receive additional benefit from amounts beyond 50% NH_4-N, those treated with FWD continued to increase biomass at the 100% NH_4-N rate, as can be observed in the in the regression curve in Figure 3.3.



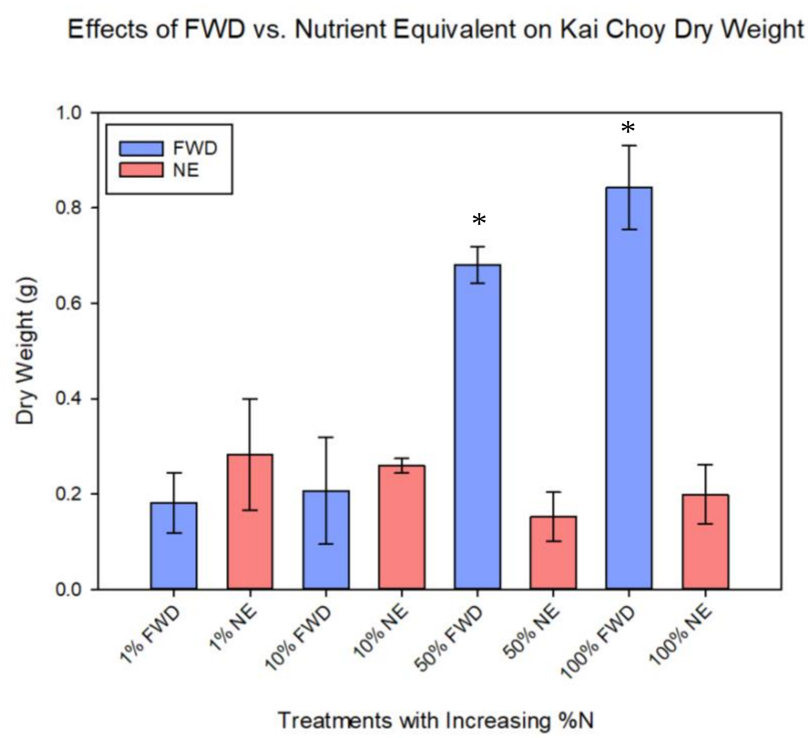


*Figure 3.1: Dry weight of kai choy with digestates vs. nutrient equivalents for both LCD (above) and FWD (below). * indicates significant differences between digestate and nutrient equivalent.*

Regression analysis showed a significant effect of the interaction term between amount of N and nutrient source for both digestates ($p=0.014$ for LCD and $p<0.001$ for FWD). This indicated that plant response to increasing nitrogen was different for digestates vs. nutrient solutions. Regression analysis for LCD showed a quadratic-like response (Fig 3.2, R-Sq(adj) = 0.641, $p=0.003$) of dry weight to digestate addition, with a plateau in biomass with additional digestate addition beginning between 50% and 100% of applied nitrogen. There was no response of dry weight to nutrient solution addition (R-Sq(adj) = 0.000).

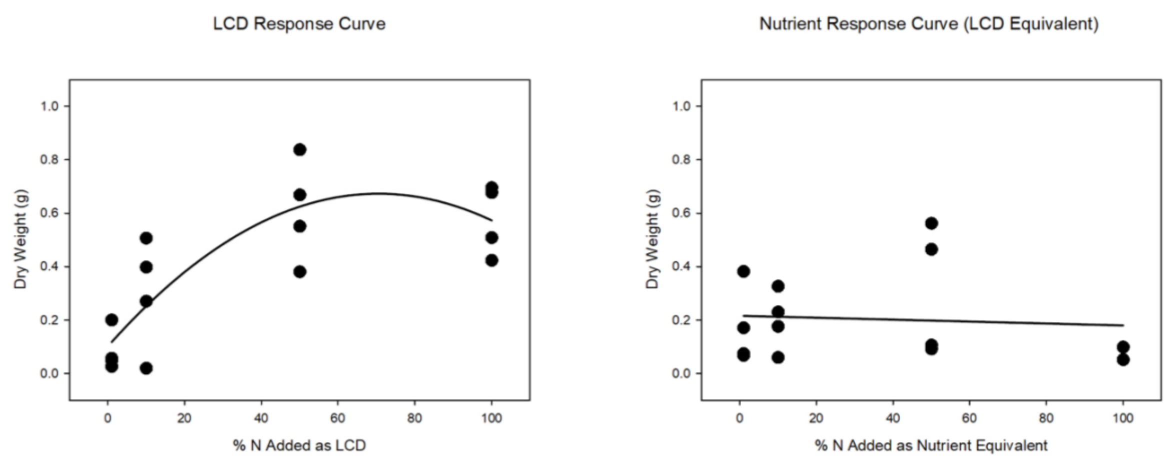


Figure 3.2: Regression response curves for LCD (left) and nutrient equivalent (right).

Slightly different results were seen for FWD and its nutrient equivalent as seen in Figure 3.3, with a significant quadratic response of dry weight to FWD addition (R-Sq(adj) = 0.825, $p<0.001$). However, the threshold at which the addition of FWD no longer increases dry biomass may be at or just beyond the rates applied in this experiment (i.e. greater than 100% N applied). Again, there was a negligible response to nutrient solution addition (R-Sq(adj) = 0.004).

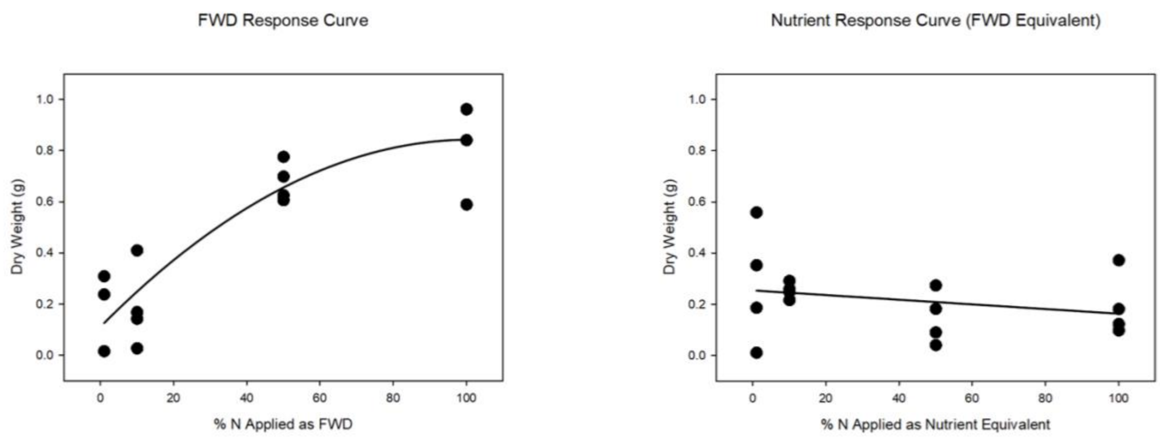


Figure 3.3: Regression response curves for FWD (left) and nutrient equivalent (right).

3.2.2 Plant Biomass with Digestates vs. Mineral Solutions

In order to further test whether the nutrients alone were causing improved plant growth with digestates, an additional control of MiracleGro was included at the 100% nutrient rate. Although not statistically significant, plants grown with 100% food waste digestate produced the highest dry biomass on average at 842.5 ± 87.6 mg as seen in Table 3.2. When we compared plant growth between MiracleGro treatments at 100% and digestate treatments at both 50% and 100% of the fertilizer rate, there was no significant difference ($p = 0.658$).

Table 3.2: Treatment effects on dry weight of kai choy.

Treatment	**Dry weight (mg)**	**SE Mean**
MG 100%	732.0 A	253
FWD 100%	842.5 A	87.6
FWD 50%	681.0 A	38.5
LCD 100%	581.3 A	65.8
LCD 50%	614.2 A	96.1
p-value	0.658	

Different letters indicate significant differences between treatments.

3.3 Discussion

In this experiment, dry plant biomass increased with increasing rate of digestate addition, but not with increasing amounts of equivalent nutrient solutions. The significant difference in plant growth response to these different treatments strongly suggests that there are effects of digestate beyond nutrients. Both LCD and FWD had relatively high concentrations of Na^+ and Cl^- and high electrical conductivity (EC), as did their corresponding nutrient solutions. However, digestates, unlike their nutrient equivalents, also contained organic matter and microbes, which can provide multiple benefits to soil and plant growth and may have been responsible for improved plant growth in this experiment.

Organic substances can act as chelators for micronutrients, making them more soluble, and thus more plant-available (Clemens et al, 1990). Organic matter can also help buffer pH in soils, reducing potentially harmful effects of extreme pH. Of particular interest for this study, organic matter can improve soil properties and increase plant growth and yield in high salinity soils (Diacono and Montemurro, 2015). Because the digestate itself contains organic matter, these beneficial impacts may be mitigating the otherwise potentially harmful effects of high EC and high levels of Na and Cl found in the digestates. In addition, the presence of microorganisms in digestates may be benefiting plants grown with them, effectively acting as plant growth promoting rhizobacteria and fungi (PGPR). Specifically, PGPR can promote nutrient cycling and reduce the effects of abiotic stresses, including high salinity, on plants through a variety of different mechanisms (Friesen et al., 2011; Qin et al., 2016). Since the nutrient solutions contained no organic matter and were not inoculated with microorganisms, plants treated with nutrient solutions would presumably be negatively affected without the associated benefits of organic matter and microorgansims that would be expected in digestates.

There was no significant increase in growth with increasing rates of either equivalent nutrient solution, even though increasing amounts of important plant nutrients, such as NH_4-N, were being added. This suggests that the growth of plants in these treatments was limited by something else in the nutrient solutions, most likely the high salinity. Conversely, there was an increase in plant growth with increasing addition of digestate. The same amount of salts were added with digestates as with the corresponding nutrient solutions. This suggests either mechanisms that improve growth enough to counteract the possible negative effects or ones that actually reduce the negative effects observed with mineral solutions or a combination of both. As mentioned above, the addition of organic matter and/or microbes in digestates could be effectively decreasing the harmful effects of high salinity and EC, thus allowing plants to more fully utilize the available nutrients in order to grow.

To our knowledge, the only other study in the literature that has used a similar approach, in which the fertilizer treatment received the same proportions of all macronutrients as well as Na^+ and Cl^- is one by Gunnarsson et al (2010). They did not observe similar trends in which there were negative effects of nutrient solution addition contrasting with strong positive effects of digestate and actually saw slightly improved growth with the nutrient solution. However, they were working with ryegrass, which can tolerate an EC of up to 5 mS/cm, making it less sensitive than other Brassicaceae, including *Brassica rapa*, which can experience yield loss beginning at an EC of 3.2 (Marcum, 2006 and Shannon and Grieve, 1999).

High salinity could also help explain the different responses of plants to increasing amounts of LCD vs. FWD. Because the LCD was lower in nitrogen, more of it was needed in order to satisfy the N requirements. Along with increasing N, increasing amounts of salts were added that may have contributed to the declining biomass of plants treated with 100% LCD. Previous research suggests the possible negative effects of some digestates at higher rates of application due to their high EC (Abdullahi et al., 2008; Alburquerque et al., 2012).

Digestates increased yields in comparison with mineral solutions by more than 100% at both 50% and 100% of required N. In addition, the similar plant growth response to MiracleGro and digestates at both 50% and 100% fertilizer rate in this early growth experiment suggest that digestates may be able to supplement or partially replace synthetic fertilizers. However, because of the potential harmful effects of high EC, Na^+ and Cl^- in digestates, more research is needed to confirm optimal rates of application that minimize any harmful effects while maximizing yields.

Chapter 4.

Assessing the Effects of Digestates and Combinations of Digestates and Fertilizer on Yield, Root Growth and Nutrient Use of Kai Choy

4.1 Introduction

As the human population continues to grow, agriculture must address increasing challenges. Farming systems must adapt to changing conditions in order to produce sufficient food for the growing population, and at the same time must consider their environmental impact. Efforts to reduce industrial inputs to agricultural systems by replacing them with renewable and/or recycled resources can help reduce pollution by chemical fertilizers while also helping to reduce the overall footprint of farming systems (Borges de Oliveira et al, 2011; Insam et al, 2015).

Anaerobic digestion is a multiple yield process that produces both energy in the form of biogas and fertilizer in the form of digestate. While often seen as a waste or by-product of the anaerobic digestion process, digestate must be considered as another valuable resource. Digestate helps recycle valuable nutrients back into agricultural systems, often in a more plant-available form than in raw materials (Plaixats et al, 1988). Digestate is particularly rich in ammonium nitrogen (NH_4-N), a form of nitrogen that is readily available for uptake by plants. As such, much of the research on digestates has focused on its value as a nitrogen source (Möller and Müller, 2012). Digestate also contains organic matter which has multiple benefits in agriculture including increasing microbial activity and nutrient cycling (Alburquerque et al., 2012). In addition, bioactive substances such as phytohormones and hormone-like compounds have been found in digestates (Kostenberg et al, 1995; Liu et al, 2009; Scaglia et al, 2015; Li et al, 2016). However, the effects of digestates on plant growth are variable, with some studies showing reduced growth with digestates, others showing improved growth and yet others showing no improvement in growth (Möller and Müller, 2012; Nkoa, 2014). These differences arise from a variety of sources including: the plant species used (Gunnarsson et al, 2010; Nkoa, 2014; Tsachidou et al, 2019), digestate composition (Tampio et al, 2010; Alburquerque et al, 2012), environmental conditions (Nabel et al, 2017), production system (Möller and Müller et al, 2012; Wang et al, 2019), and the control used in the experiment (i.e. unamended vs. commercial fertilizer control).

Much of the research on digestates focuses on the effects on aboveground biomass as it translates into yield. However, roots are integral for plant growth and nutrient acquisition and changes in root morphology can in turn have significant effects on nutrient use efficiency and yield. Root growth and morphology can be significantly altered by addition of fertilizers and amendments. The limited research on the effects of digestates on root growth shows varying and somewhat contradictory effects on overall root biomass. Some studies showed increased root biomass with digestate treatment when compared with either an unamended control or mineral fertilizer (Garg et al, 2005; Gunnarsson et al, 2010). Others showed decreased root biomass with digestate use (Van Eekeren et al, 2009; Andruschkewitsch et al, 2013), and yet others showed no difference in root biomass with digestate compared with mineral fertilizers (Wentzel and Joergensen, 2016). The seemingly contradictory nature of these results could be due to differences in digestate composition, plant species and/or experimental conditions.

Digestate addition may also induce important changes in root growth rate and root morphology that affect nutrient use and growth. These effects may not be captured by biomass measurements alone and may have significant effects on plant yield, potentially even leading to overall biomass differences. Specifically, early root growth and changes in root hairs can have important implications for overall plant growth and yield. Especially during early growth stages, increased root density can be important for plant establishment and growth (Wang et al, 2016).

Digestate is a complex mixture containing nutrients as well as various organic compounds. Previous studies have found various phytohormones including auxin and auxin-like compounds in digestates (Kostenberg, 1997; Scaglia et al, 2015; Li et al, 2016). Although many phytohormones are involved in root hair development, auxin is the most well-studied for its role in root hair elongation. Auxin plays an organizing role in root hair development both by directly affecting root hair elongation and by mediating the effects of other hormones and compounds (Pitts et al, 1998). Increases in root hair length and density contribute to increased nutrient use efficiency (NUE) by plants due to an increased surface area in contact with the soil (Baligar et al, 2001). This increased NUE translates for both mobile and immobile nutrients but may be more pronounced with immobile nutrients such as phosphorous. This increased potential for nutrient acquisition may subsequently lead to increased plant growth and yields beyond the addition of nutrient fertilizer alone (Baligar, 2001). Root hairs are particularly important in the acquisition of nutrients that are low-mobility, high-demand nutrients in plants such as phosphorous, and can be responsible for up to 80% of the total uptake of certain nutrients (Jungk, 2001). Ultimately, stronger and more extensive roots can lead to an increase in primary productivity and aboveground yield (Ertani et al, 2009). Significant positive relationships have been found between root surface area and micro- and macronutrient content of shoots (Lee and Cho, 2013).

Much of the research on nutrient use efficiency and nutrient uptake with digestates focuses on nitrogen (N), the primary macronutrient required for plant growth. Digestate contains a large portion of its N as ammonium (NH_4), which is a mineral form of nitrogen that is much more readily accessible to plants than the organic nitrogen likely contained in the undigested materials. Previous research has shown similar fertilizer values for digestates and mineral fertilizers when they are applied based on the NH_4-N rather than total N, suggesting that this mineral N is the most important form for short-term fertilization (Möller and Müller, 2012). However, it is important to note that digestates also contain organic N, which gradually mineralizes and becomes available over subsequent cropping cycles (Gunnarsson et al, 2010). Tsachidou et al (2019) found a lower NUE for digestates than chemical fertilizers at lower rates of fertilization while there was a similar or slightly higher NUE at higher rates of fertilization. Gunnarsson et al (2010) found lower dry mass production and lower N uptake with digestate treatment than chemical fertilizers in the early growth stages of rye grass, and no difference after 136 days of growth. Wentzl and Joergensen (2016) showed an increase in plant yield with increasing NH_4-N applied as digestate up until a certain point, and then a subsequent decrease, suggesting a decrease in RE at higher rates of application. There are relatively few studies directly investigating the effects of digestate on phosphorus (P) availability and uptake, and the majority of those that do compare it with undigested materials, rather than with mineral fertilizers. While there is some suggestion of increased P availability from digestates as compared with raw manures (Massé et al, 2011; Alburquerque et al, 2012), there are some that suggest there is no effect of digestates on P availability as compared with manures (Bachmann et al, 2014; Möller and Stinner, 2010). However, P availability depends greatly on the pH of the digestate and may be unavailable due to high pH (Möller and Müller, 2012). Overall, nutrient use efficiency from digestates varies based on the rate of fertilization and length of cropping cycle, as well as digestate composition, thus making it important to understand dynamics at appropriate levels of fertilization for both soil and crop types to optimize digestate use.

While digestates have multiple beneficial attributes for plant growth, there are important and potentially negative aspects of using digestates as fertilizers. Specifically, the high electrical conductivity (EC) of many digestates can make them unsuitable as sole fertilizers, and may instead be better used in combination with other fertilizers, particularly with more sensitive plants (Alburquerque et al, 2012; Wang et al, 2019). Maunuksela et al (2012) found increased or similar growth with barley using digestates as compared with mineral fertilizers, but decreased Chinese cabbage growth, suggesting that full-strength digestate may be too saline for salt-sensitive crops. Two studies have found that replacing some fertilizer with digestate can produce similar or greater plant growth than fertilizer alone, likely due to the moderation of EC (Zhang et al, 2017; Wang et al, 2019).

Wang et al (2019) conducted a study assessing the effects of poultry litter digestate and combinations of digestate and mineral fertilizer on hydroponic lettuce production. They found that replacing ½ of the commercial nutrient solution with poultry digestate produced similar yields as nutrient solution alone. The combined treatment also yielded greater root biomass as well as root length, volume and number of root tips than did the Hoagland nutrient solution alone. When using full-strength digestate, they saw a significant reduction in growth, likely attributable to the high EC of the digestate. Zhang et al (2017) found similar results when combining digestate with mineral fertilizer in rice production. Both studies suggest that digestate may be best utilized as a complement to, rather than substitute for, mineral fertilizers in order to optimize growth while minimizing synthetic inputs.

While there are quite a few studies that investigate the effects of digestates in grain crops and grasses, especially barley and ryegrass, there are relatively few studies on the on shorter term vegetable crops and leafy greens (Möller and Müller, 2012). Even within grassland systems, research has shown different plant responses to digestate application both in different plant species and with different digestates, suggesting the need for both species- and digestate-specific studies to best predict plant response (Andruschkewitsch et al, 2013). In addition, there are a few studies suggesting that digestates may be better applied in combination with fertilizers than on their own in order to mitigate possible negative effects of digestates (Riva et al, 2016; Zhang et al, 2017; Wang et al, 2019). There is a lack of information on how digestates affect plant growth and nutrient status when combined with fertilizers, and the information available is either in grassland or hydroponic systems, both quite different from annual vegetable systems (Tsachidou et al, 2019; Wang et al, 2019). Moreover, the majority of research with digestates focuses on its effects on aboveground biomass and yield, but many suspected mechanisms for digestates' benefits to growth, including bioactive compounds and phytohormone activity, have to do with root growth (Ertani et al, 2013; Scaglia et al, 2015; Li et al, 2016; Scaglia et al, 2017). More research is needed to better understand if and how digestates affect root growth, and how that might in turn impact nutrient uptake and use as well as overall biomass and aboveground yields.

Given the uncertainties associated with digestate's effects on root growth and nutrient use when combined with fertilizers, our study addressed the following objectives:

1) To evaluate the effects of two different digestates, a lignocellulosic digestate (LCD) and food waste digestate (FWD), both alone and in combination with a mineral fertilizer, on plant growth, including aboveground biomass, root growth and root hair density and length.
2) To evaluate the effects on nutrient uptake and use efficiency with respect to nitrogen (N), phosphorus (P) and potassium (K), with a particular focus on N.
3) To evaluate potential negative effects of digestate, including biohazard analysis and salinity.

With respect to the first objective, FWD and combinations of either digestate and fertilizer were hypothesized to improve plant aboveground biomass over the mineral fertilizer control by providing higher amounts of NH_4-N with less applied digestate, and thus reducing negative effects of salinity and also providing benefits to root growth. Digestates were hypothesized to increase root length and root hair density as a response to bioactive substances in digestates. For the second objective, nutrient uptake and use efficiency were hypothesized to improve with increased root growth and root hair density as nutrient acquisition is related directly to root morphology. Lastly, increased salinity, particularly due to high Na^+ and Cl^-, is hypothesized to be an important negative effect of digestate addition and can be best managed by combining digestates with mineral fertilizers to obtain optimum benefits while minimizing negative effects on plant growth.

4.2 Materials and Methods

4.2.1 Experimental Growing Conditions

The experiment was conducted in the Pope Greenhouse facility at the University of Hawaii at Manoa from February 14 – March 25, 2019 for a total of 40 days of growth. We used *Brassica juncea* (var. Hirayama) as the test crop due to its rapid growth and relative heat tolerance. The experiment was conducted as a randomized complete block design and plants were grown in rhizoboxes on a single greenhouse bench as shown in Figure 4.1.

Figure 4.1: Empty rhizobox (A) and rhizoboxes arranged in RCBD in greenhouse (B).

Each rhizobox consisted of an opaque plastic back and a clear plexiglass front that was covered by foil at all times except for imaging to limit UV exposure to roots. A single piece of black felt was placed along the clear plane to increase contrast for imaging. The boxes were filled with potting medium behind the felt. Rhizoboxes were kept at a 45˚ angle with the clear plane facing downwards throughout the growing period to encourage root growth along the clear plane.

Sunshine Mix # 1 (Sun Gro Horticulture, Agawam, MA) was pre-mixed with water to reach 80% of field capacity and calcium sulfate ($CaSO_4 \cdot 2H_2O$) at a rate of 2 g/kg to ensure adequate calcium for plant growth in all treatments. Rhizoboxes were filled with this mixture at a rate of 177 g of oven-dried medium equivalent. Five *Brassica juncea* seeds were sown directly into each rhizobox container and thinned to a single plant after 5 days of growth. The first treatment solutions were mixed with water to bring boxes to field capacity and added immediately after seeding. Treatments were subsequently applied every 10 days throughout the growing period for a total of 4 applications. Plants were harvested 40 days after seeding.

4.2.2 Characterization of Fertilizer and Digestates

The liquid fertilizer used in this experiment was GrowBig® (6-4-4, with micronutrients) from FoxFarm (Samoa, CA). The digestates used in this experiment were produced in the Khanal laboratory at the University of Hawaii. The lignocellulosic digestate (LCD) was produced from napier grass (*Pennisetum purpureum*, a grass grown specifically for the production of biogas) via semi-continuous anaerobic digestion. The food waste digestate (FWD) was produced from food waste collected from the UH Manoa cafeteria and produced via semi-batch anaerobic digestion. The conductivity (EC) were measured for the digestates with an Orion DuraProbe (Thermo Scientific, Waltham, MA). The chemical properties of the digestates are shown in Table 4.1.

Table 4.1: Characteristics of the digestates and fertilizer.

Parameter	***Lignocellulosic Digestate****	***Food Waste Digestate****	***FoxFarm GrowBig®****
Total Solids	2.87%	1.21%	0%
Electrical Conductivity (mS/cm)	8.34	10.99	**
pH	8.4	8.6	3.24
Total Nitrogen (mg kg^{-1} ww)	710	1790	60,000
Ammonium Nitrogen (mg kg^{-1} ww)	270	1150	29,000
Organic Nitrogen (mg kg^{-1} ww)	440	640	0
Nitrate Nitrogen (mg kg^{-1} ww)	0	0	31,000
Phosphorus (mg kg^{-1} ww)	120	460	17,500
Potassium (mg kg^{-1} ww)	920	380	33,200
Calcium (mg kg^{-1} ww)	90	250	0
Magnesium (mg kg^{-1} ww)	110	130	6000
Sulfur (mg kg^{-1} ww)	70	410	**
Sodium (mg kg^{-1} ww)	910	930	230
Chloride (mg kg^{-1} ww)	2500	1300	0
Iron (mg kg^{-1} ww)	29	496	1000
Zinc (mg kg^{-1} ww)	0.9	13.1	500
Copper (mg kg^{-1} ww)	1	3.7	500
Manganese (mg kg^{-1} ww)	1	1	500

** indicates full strength or concentrated form. ** indicates unknown quantity.*

4.2.3 Treatments

The application rate for treatments was normalized based on the amount of mineral nitrogen (N) applied. Fertilization was based on nitrogen recommendations of 56 kg ha^{-1} for leafy greens grown in organic soils (Hochmuth et al, 1994). This amount was then doubled to ensure adequate nutrition for the treatments receiving 100% of the recommended fertilizer N. Therefore, the 100% nutrient rate was established at 518 mg/kg N based on a depth of 15 cm and a bulk density of 0.144 g cm^{-3}. Ammonium N was considered as the only nitrogen source in digestates since shorter growing periods have been shown to produce little to no mineralization of organic N in previous studies with digestates (Gunnarsson et al, 2010).

Treatments were divided into two nutrient rates, 50% and 100% of required nitrogen, as well as a 0% control. Treatments consisted of varying combinations of each digestate and fertilizer to reach the appropriate level of nitrogen. Plants receiving 100% of the nutrient level were fertilized with 91.7 mg N per pot containing 177 g of oven-dried media equivalent.

Percentages recorded indicate the percent of the 100% rate of nitrogen applied with each amendment and are specified in Table 4.2 below. The fertilizer control consisted of 0% digestate and 100% fertilizer (W0F100). The unamended control (W0F0) was treated with water and no additional nutrients were applied. There were a total of 13 treatments and 5 blocks for a total of 65 experimental units.

Table 4:2 Treatment specifications.

Treatment	Description	% N	% Digestate	% Fertilizer
W0F0	Unfertilized control	0	0	0
W0F50	50% fertilizer control	50	0	50
F10F40	10% FWD, 40% fertilizer	50	10	40
F50F0	50% FWD	50	50	0
L10F40	10% LCD, 40% fertilizer	50	10	40
L50F0	50% LCD	50	50	0
W0F100	100% fertilizer control	100	0	100
F10F90	10% FWD, 90% fertilizer	100	10	90
F50F50	50% FWD, 50% fertilizer	100	50	50
F100F0	100% FWD	100	100	0
L10F90	10% LCD, 90% fertilizer	100	10	90
L50F50	50% LCD, 50% fertilizer	100	50	50
L100F0	100% LCD	100	100	0

4.2.4 Plant Growth Analyses

At harvest, plants were cut just above soil level to separate shoots from roots. Shoots were immediately weighed for fresh weight and roots were cleaned thoroughly and blotted dry before weighing for fresh weight. Both roots and shoots were oven-dried at 65˚C until they maintained a constant weight to obtain dry weight.

4.2.5 Root Length Analysis

In order to assess differences in root growth, we took digital photographs of the clear plane of the rhizoboxes every 3-4 days during the active growth period for a total of 7 photographic events. We used a Nikon D7500 digital camera (Nikon USA, Melville, NY) mounted on a tripod to capture images. Photographs were then standardized by cropping each image to a 15x15 cm square in the center of each rhizobox in order to minimize edge effects. Image analysis on roots was started 18 days after seeding, hereafter referred to as time point 1. This was chosen as the starting point because at this point roots were observed to be consistently within the center of the square for all treatments. The 225 cm^2 area was then analyzed at each of the 7 time points for total root length using imageJ (Schneider et al, 2012). We used the grid intersect method (Newman, 1966) to estimate root length based on the number of intersections between roots and the lines of a randomly oriented 1-cm^2 grid. Briefly, all intersections between roots and lines of the grid were counted and converted into root length using equation 4.1:

$$R = \frac{\pi N A}{2H} \qquad [4.1]$$

where R is total root length, N is number of intersections between the root and straight lines of the grid, A is the area of the grid, and H is the total length of straight lines within the grid.

4.2.6 Root Hair Analysis

Subsamples of roots were collected at harvest and analyzed for root hair length and density using a ranking system (Vieira et al, 2007). In brief, three to five root subsamples were collected from each rhizobox at harvest and stained overnight in acid fuschin stain. These roots were viewed under a dissecting scope mounted with a digital camera and photographed at 15x magnification. Root images were visually sorted using a rating scale of 1-9 based on both length and density as follows: 1 = very few root hairs; 3 = between 1 and 5; 5 = intermediate root hair density/length; and 9 = abundant root hairs. Calibration images are shown in Figure 4.2. The rankings for all subsamples were averaged to obtain a value for each experimental unit.

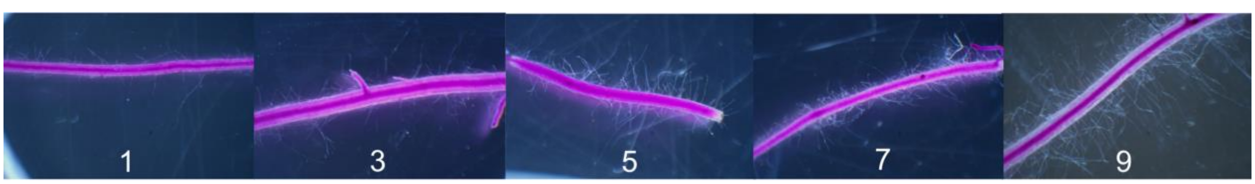


Figure 4.2: Root hair calibration images.

4.2.7 Plant Nutrient Analysis

All above ground parts were ground and sieved to 250 μm after oven drying. Samples were weighed on a microbalance before N analysis. Following dry ashing and acid digestion, they were run on a Carlo Erba NC 2500 Elemental Combustion System/Pneumatic Autosampler (CE Elantech, Inc., Lakewood, NJ) for weight percent nitrogen. All other macro and micro nutrients were measured by DairyOne forage lab. Following dry ashing and acid digestion, samples were analyzed by ICP using a Thermo iCAP 6300 Inductively Coupled Plasma Radial Spectrometer (Thermofisher Scientific, Waltham, MA). This data was then used to calculate the apparent recovery efficiency (RE), agronomic efficiency (AE), and physiological efficiency (PE) for nutrients of interest using equations 4.2-4.4 (Fixen, 2015):

$$RE\ (\%) = (U - U_0)/F \qquad [4.2]$$

$$AE = (Y - Y_0)/F \qquad [4.3]$$

$$PE = (Y - Y_0)/\,(U - U_0) \qquad [4.4]$$

Where U is nutrient uptake in aboveground biomass (concentration X dry weight) with nutrient applied; U_0 is nutrient uptake in aboveground biomass with no nutrient applied; F is amount of nutrient applied; Y is yield of harvested portion of crop with nutrient applied; and Y_0 is yield of harvested portion of crop with no nutrient applied.

These three parameters address slightly different questions with respect to nutrient use under the different treatments. Recovery efficiency indicates how much of the nutrient applied the plants took up. Agronomic efficiency quantifies the benefit in productivity from the applied nutrient. Physiological efficiency shows the relative ability of plants to transform nutrients from the source into yield.

4.2.8 Electrical Conductivity and Biohazard Analysis

Electrical conductivity was measured using the 1:2 (v:v) soil:water extract method (Dellavalle, 1992). We added 20 cm^3 of potting media from the selected treatments and 40 mL of distilled water to a falcon tube. The tubes were shaken for 30 seconds every hour for four hours and then allowed to equilibrate for 24 hours. After 24 hours, electrical conductivity (EC) was measured with an Orion DuraProbe 4-electrode conductivity cell (Thermo Scientific, Waltham, MA) for each 1:2 extract ($EC_{1:2}$). The $EC_{1:2}$ values were converted to saturated paste values (EC_{SS}) in order to evaluate phytotoxicity. We used equation 4.5 below, adapted from Sonneveld and Voogt (2009) and omitted the term for SO_4 because of the negligible amount present in the media:

$$EC_{SS} = 0.908dEC_{1:2} - 0.89d + 0.68 \qquad [4.5]$$

where *d* is the dilution factor, a ratio between the water content of the 1:2 suspension and the water content of field moist soil, determined in a previous experiment as 2.52.

Fecal coliforms were quantified both in the fresh digestates and in media samples collected from each pot at harvest. Fecal coliforms were enumerated using the multiple tube fermentation technique (EPA, 9221b,e).

4.2.9 Statistical Analysis

Statistical analyses were performed in both R (R Core Team, 2013) and Minitab 18 (State College, PA). For all analyses, a *P* value was considered significant at the 0.05 level. Blocks were treated as replicates and the blocking effect was accounted for in all models. Models were tested for assumptions of normality and equal variance. For all parameters described, we conducted a one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference test (HSD) at the $\alpha = 0.05$ level of significance in Minitab. Regression analysis with a General Linear Model in Minitab was used to analyze response of growth parameters to increasing amounts of digestate at the 100% fertilization level. In addition, we analyzed root growth data over time using repeated measures analysis for the 100% nutrient levels in R. We compared the least squares means (LS means) between treatments over time using Tukey's HSD. Treatments at individual time points of interest were then further analyzed using ANOVA and Tukey's HSD in Minitab. All measurements of variability and error bars in graphs are standard error of the mean.

4.3 Results

4.3 1 Plant Growth

Shoot dry biomass increased with increasing levels of fertilization (Fig 4.3). All treatments produced greater yields than the unamended control ($p<0.001$). Biomass production was significantly lower in all 50% N treatments, so analyses were conducted on treatments at the 100% N rate. All labels in graphs represent the percent of N satisfied by that fertilizer, LCD, FWD or mineral fertilizer (MF). In digestate treatments less than 100%, mineral fertilizer was added to satisfy the 100% N requirement.

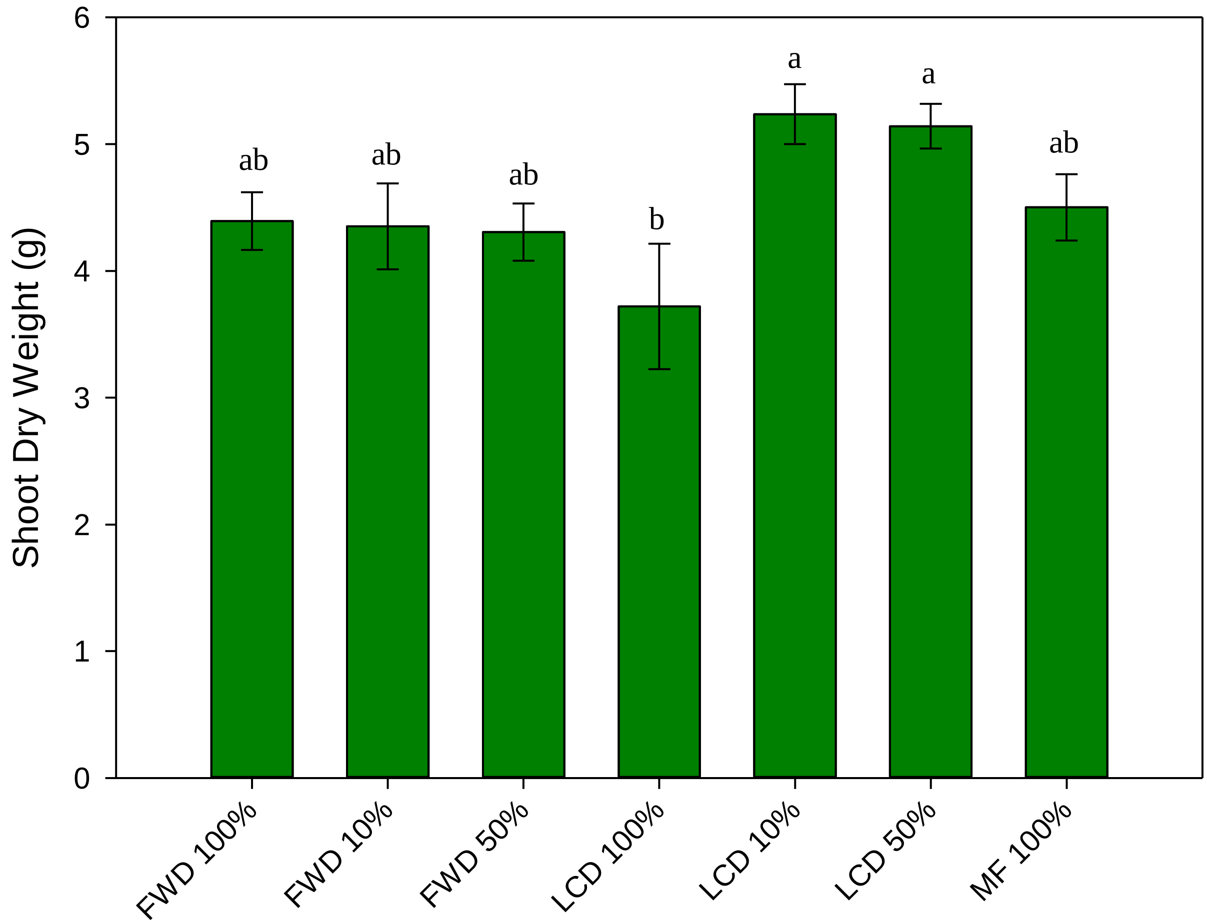


Figure 4.3: Shoot dry weight by treatment. Percentages are % of total N satisfied by that fertilizer. The remainder is mineral fertilizer. Error bars are SE of the mean. Means that do not share a letter are significantly different.

Analysis of variance at the 100% fertilization rate showed significant differences between treatments (p=0.028). This difference was largely driven by low biomass production in the 100% LCD treatment. The treatments containing mixtures of lignocellulosic digestate and fertilizer (LCD 10% and LCD 50%) produced the highest biomass of all of the treatments, although not significantly different from the fertilizer control.

Regression analysis for treatments at 100% N with increasing amounts of LCD showed a significant quadratic relationship between the amount of digestate added and shoot dry weight (R-Sq(adj)=0.376, p=0.004). Shoot dry weight increased with increasing amounts of digestate and then showed a tendency to decrease above 50% digestate (Fig 4.4). For FWD, however, there was no significant effect on shoot dry weight.

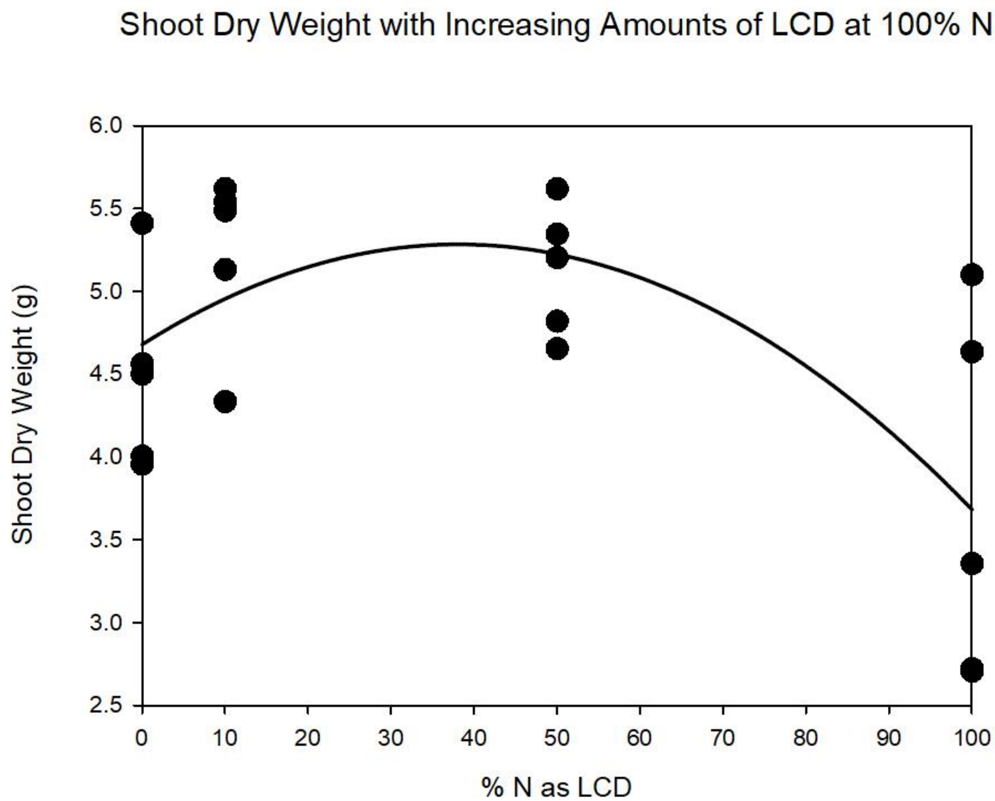


Figure 4.4: Regression curve for dry weight with increasing LCD at 100% N.

4.3.2 Total Root Length

The increase in root length over time with different treatments is shown in Figure 4.5. Root lengths diverged according to treatment as time increased. For FWD treatments (Fig 4.5A), there is a divergence as early as week 1, with the Fertilizer Control increasing plant growth more than the rest of the treatments. Subsequent divergence of specific FWD treatments from each other began after week 4. For LCD treatments (Fig 4.5B), there was a divergence as early as week 1 as well, with the pure LCD treatment increasing plant growth less than the rest of the treatments. There was a subsequent divergence after week 4 with the remainder of the treatments splitting slightly from each other.

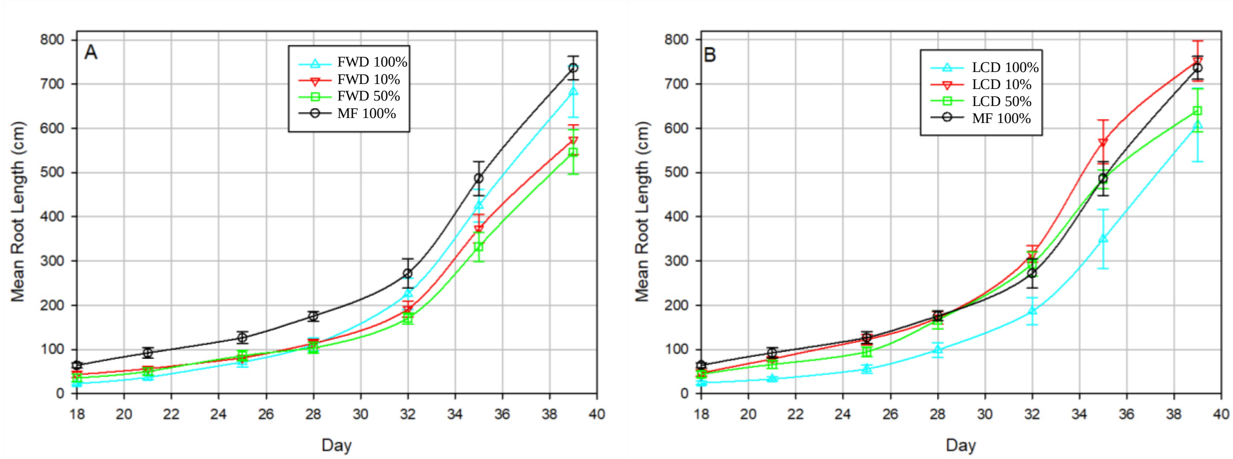


Figure 4.5: Change in mean root length (cm) over time as affected by FWD (A) and LCD (B). Error bars are SE of the mean.

There was a significant effect of treatment, time point, and the interaction of treatment and time point on root length ($p < 0.0001$, $p<0.0001$ and $p= 0.0301$, respectively). There was no significant difference between the model with mixed effects vs. fixed effects ($p=0.627$), and data was further analyzed using the fixed effects model. Least squared means (lsmeans) were significantly different between treatments averaged over time points (Fig 4.6). The greatest root lengths were in the combined LCD and fertilizer treatments (LCD 10% and LCD 50%) and the fertilizer control (MF 100%). All FWD treatments had significantly lower root lengths than the fertilizer control.

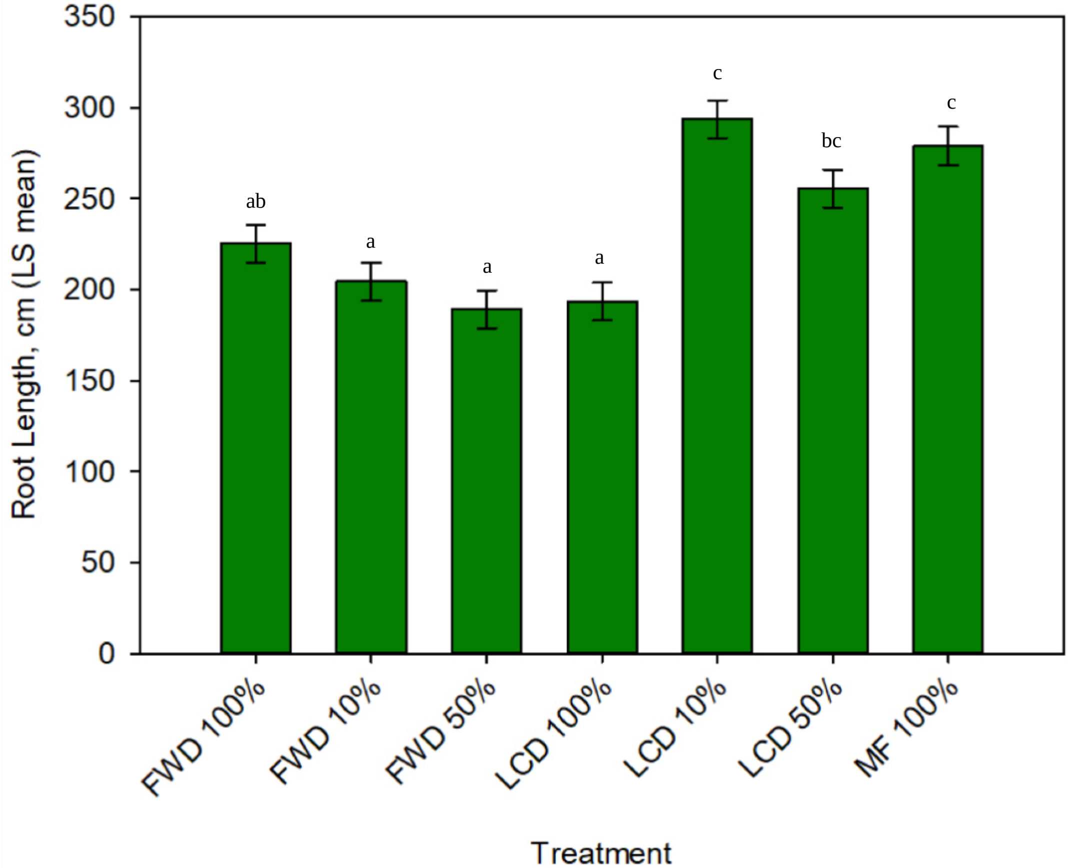


Figure 4.6: Root length LSmeans by treatment. Error bars are SE of the mean. Means that do not share a letter are significantly different.

ANOVA at individual time points of interest showed significant differences in root length due to treatment with grouping beginning at time point 1 (Table 4.3, p=0.003). At time point 1, MF 100%, LCD 10% and LCD 50% have the greatest root length, and continue to show the greatest root length throughout the growing period. Both of the pure digestate treatments showed lower root lengths than the fertilizer control. The LCD 100% treatments remained the lowest throughout the growing period. Interestingly, although treatment means continued to diverge and remain significant with increasing time up until harvest (p=0.044 at time point 7), there was no significant grouping at harvest.

Table 4:3 Mean root lengths at time point 1.

Treatment	Mean RL	SE Mean	Grouping
FWD 100%	22.93	3.29	b
FWD 10%	43.35	6.98	ab
FWD 50%	35.81	8.49	ab
LCD 100%	24.19	3.95	b
LCD 10%	46.65	6.37	ab
LCD 50%	43.67	7.11	ab
MF 100%	64.09	5.95	a

Means that do not share a letter are significantly different.

The variation in the means at harvest was likely too high to see clear differences between treatment means, although LCD 10% and MF 100% still had the greatest root lengths, and differences were visually apparent as seen in Figure 4.7. The images shown represent the lowest root length (LCD 100%) and the highest root length (LCD 10%).

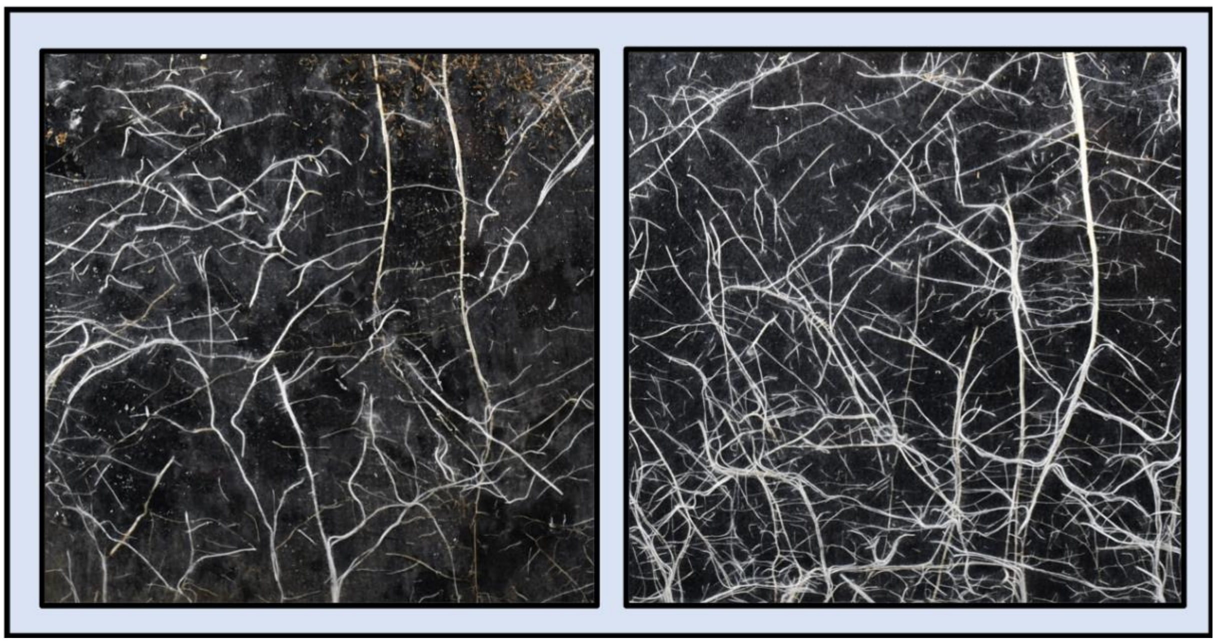


Figure 4.7: Root lengths as observed at harvest for LCD 100% (left) and LCD 10% (right).

4.3.3 Root Hairs

Although root hair length and density was highly variable, differences can be seen between treatments. A gradient of root hair length and density with different treatments can be seen in Figure 4.8.

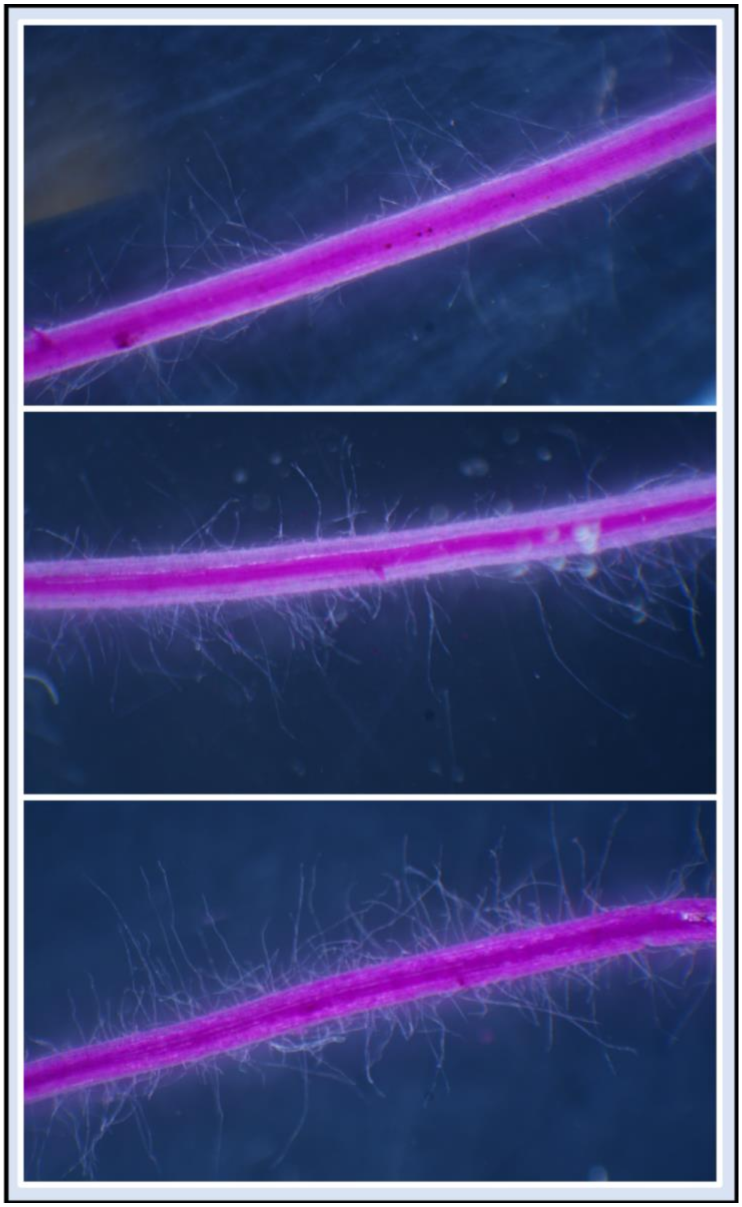


Figure 4.8: Root hair length and density variation between treatments. Increasing from top to bottom: MF100% (rating = 1), CTRL (rating = 5), LCD 100% (rating = 9).

While root hair rating was significantly different due to treatment (Fig 4.9, p=0.019), significant differences were only apparent between treatments at the 50% N level. However, treatments with LCD as the only nutrient source at both the 50% and 100% N level showed the greatest root hair length and density for their respective levels of nitrogen.

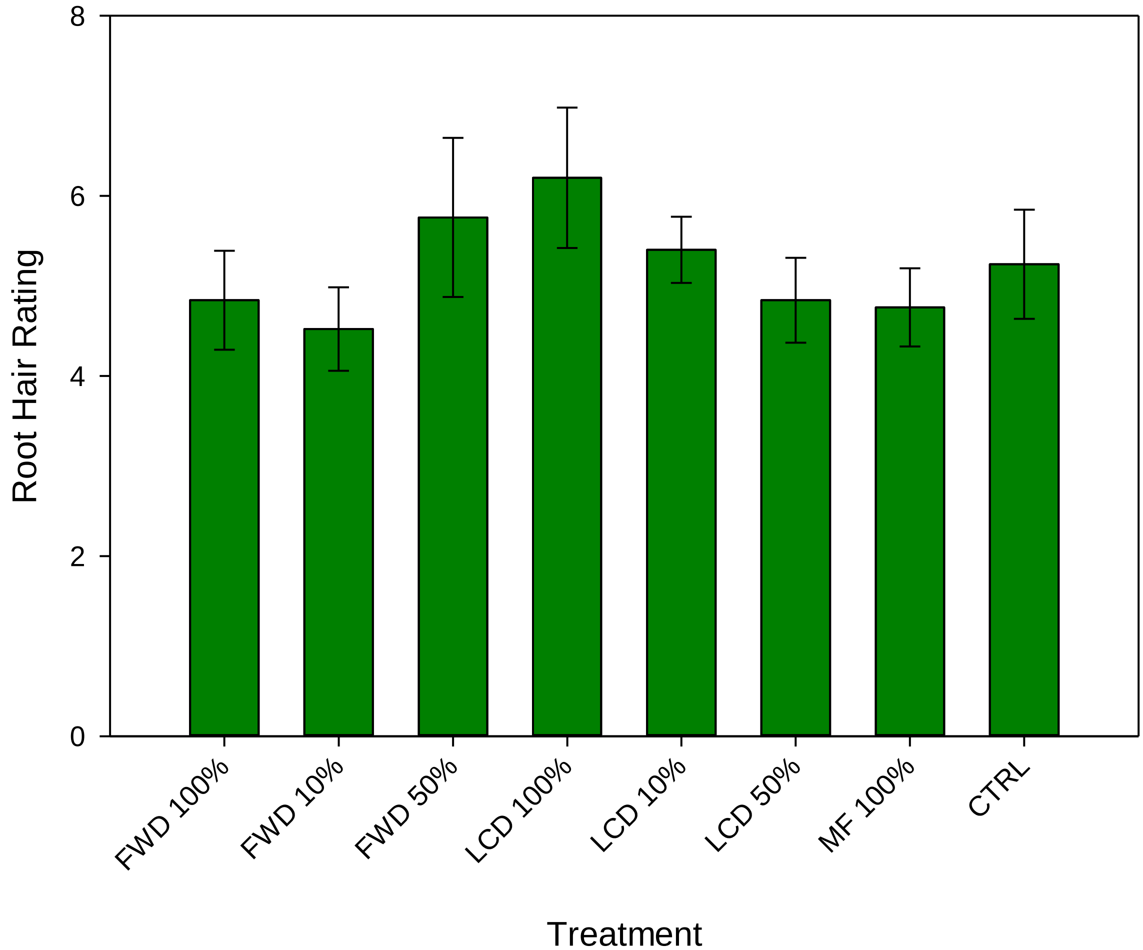


Figure 4.9: Root hair length and density for treatments at the 100% nutrient level. Error bars are SE of the mean.

4.3.4 Nutrient Uptake and Use Efficiency

There was a significant effect of treatment on both plant tissue content (%) and uptake (mg-mg^{-1}) for N, P and K (Table 4.4 and Fig 4.10, p<0.001). Nutrient uptake accounts for the amount of plant biomass as well as the percent nitrogen in the tissue, and so reflects both differences in the amount of nutrient in the tissue and the total plant biomass, while % nutrient in tissue can reflect a dilution effect and is relatively higher in plants that produce less biomass.

Table 4.4: Tissue % for N, P and K under different treatments.

Treatment	% N in Tissue		SE Mean	% P in Tissue		SE Mean	% K in Tissue		SE Mean
W0F100	1.538	CDE	0.0335	0.858	DE	0.0275	4.082	D	0.071
F100F0	1.652	BCD	0.0460	0.708	E	0.0265	4.218	CD	0.137
F10F90	1.778	BCD	0.0687	0.936	CDE	0.0518	4.652	BCD	0.253
F50F50	1.852	ABC	0.0922	0.852	DE	0.0153	4.762	ABCD	0.136
L100F0	2.174	A	0.1670	0.818	DE	0.0331	5.564	AB	0.272
L10F90	1.742	BCD	0.0751	0.844	DE	0.0374	4.57	CD	0.187
L50F50	1.750	BCD	0.0800	0.872	CDE	0.0292	5.13	ABC	0.223
W0F50	1.452	DE	0.0263	1.24	A	0.0601	4.656	BCD	0.131
F10F40	1.578	CDE	0.0532	1.242	B	0.0726	4.774	ABCD	0.0857
F50F0	1.460	DE	0.0425	0.924	CDE	0.0487	4.432	CD	0.137
L10F40	1.438	DE	0.0841	1.05	BCD	0.0532	4.714	BCD	0.21
L50F0	2.030	AB	0.1230	1.1025	BC	0.0886	5.705	A	0.454
W0F0	1.204	E	0.0108	1.564	B	0.0713	4.882	ABCD	0.128

Means that do not share a letter are significantly different.

Nutrient uptake for N, P and K can be seen in Figure 4.9. N uptake was highest in the LCD and fertilizer combinations, the treatments that also produced the greatest biomass. These treatments had a significantly higher N uptake than did both the fertilized and unamended controls. N uptake was greater than the unamended control in all amended treatments except for the 50% fertilizer control (W0F50). P uptake was highest in both the LCD and fertilizer combinations, although not significantly higher than the fertilized control. K uptake was highest in LCD and fertilizer combinations and was significantly higher in these treatments than in the fertilized control.

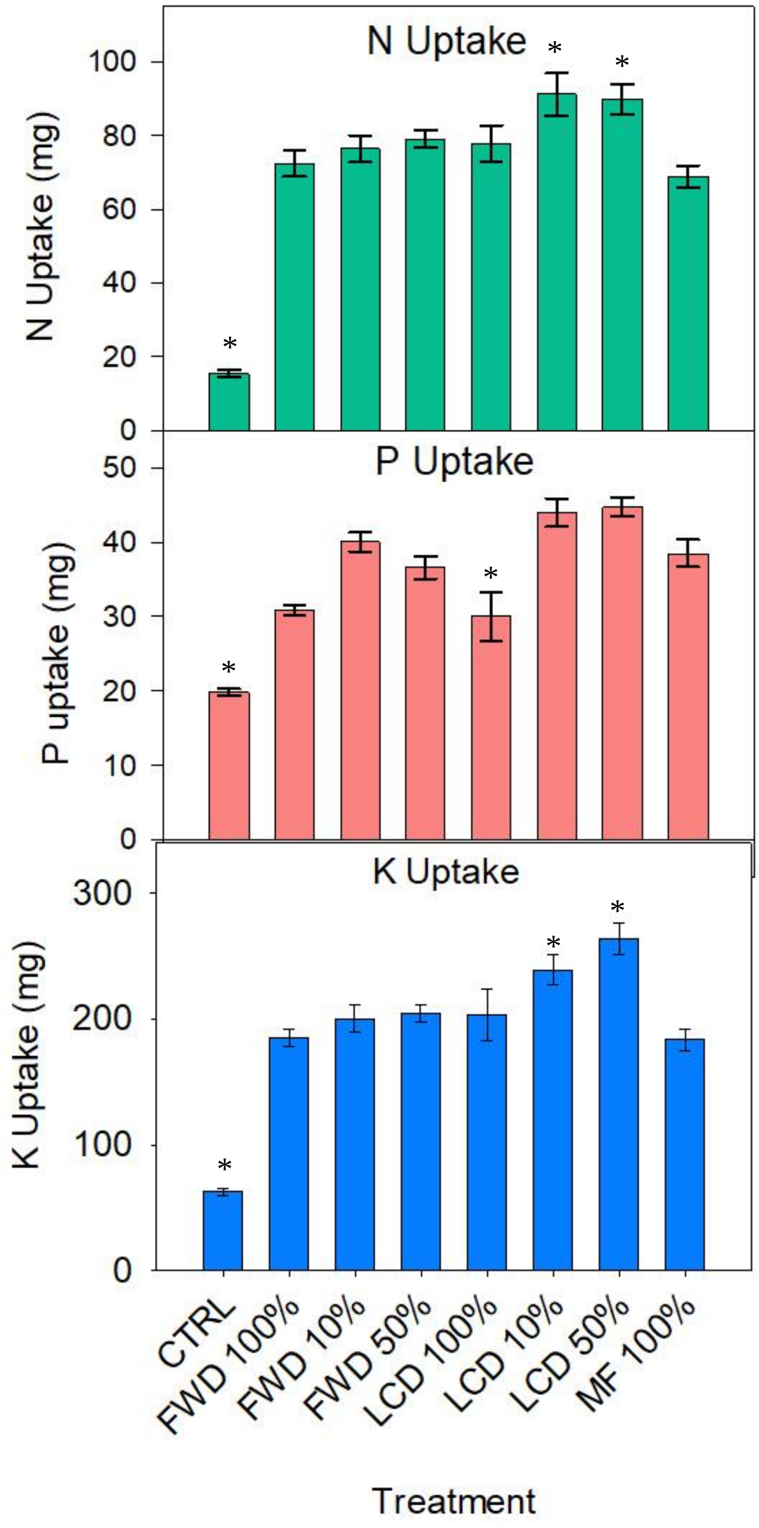


*Figure 4.10: Nutrient uptake at 100% nitrogen rate. * indicates a mean significantly different from the fertilized control (W0F100)*

Nutrient use efficiency parameters for N, P and K are all shown in Figure 4.11. The top graph in each panel is apparent recovery efficiency (RE), the middle graph is agronomic efficiency (AE) and the bottom is physiological efficiency (PE).

Apparent recovery efficiency (RE), agronomic efficiency (AE) and physiological efficiency (PE) for nitrogen were all significantly different due to treatment ($p=0.0032$, $p=0.0281$ and $p=0.0036$, respectively). RE was highest for the LCD and fertilizer combinations (LCD 10% and LCD 50%) and lowest for the fertilizer control (MF 100%). AE was highest for the LCD and fertilizer combinations and lowest for LCD 100%. PE was highest for the fertilizer control, FWD 100% and LCD 10%, and lowest for LCD 100%.

RE and AE for phosphorus were both significantly different due to treatment ($p<0.001$). Phosphorus RE and AE were highest for LCD 10%, LCD 50%, FWD 10% and the fertilizer control. Again in the case of P, the mixtures of LCD and fertilizer performed amongst the best, and the pure digestate treatments both had the lowest RE and AE of any treatments. PE was not significantly different between treatments ($p=0.0722$).

RE, AE and PE for potassium were all significantly different due to treatment ($p<0.001$). RE was highest for FWD 100% and FWD 50% and lowest for LCD 50% and LCD 100%. AE was highest for FWD 100% and lowest for LCD 100% and LCD 50%. PE was highest for pure fertilizer, FWD 100% and LCD 10% and lowest for LCD 100%.

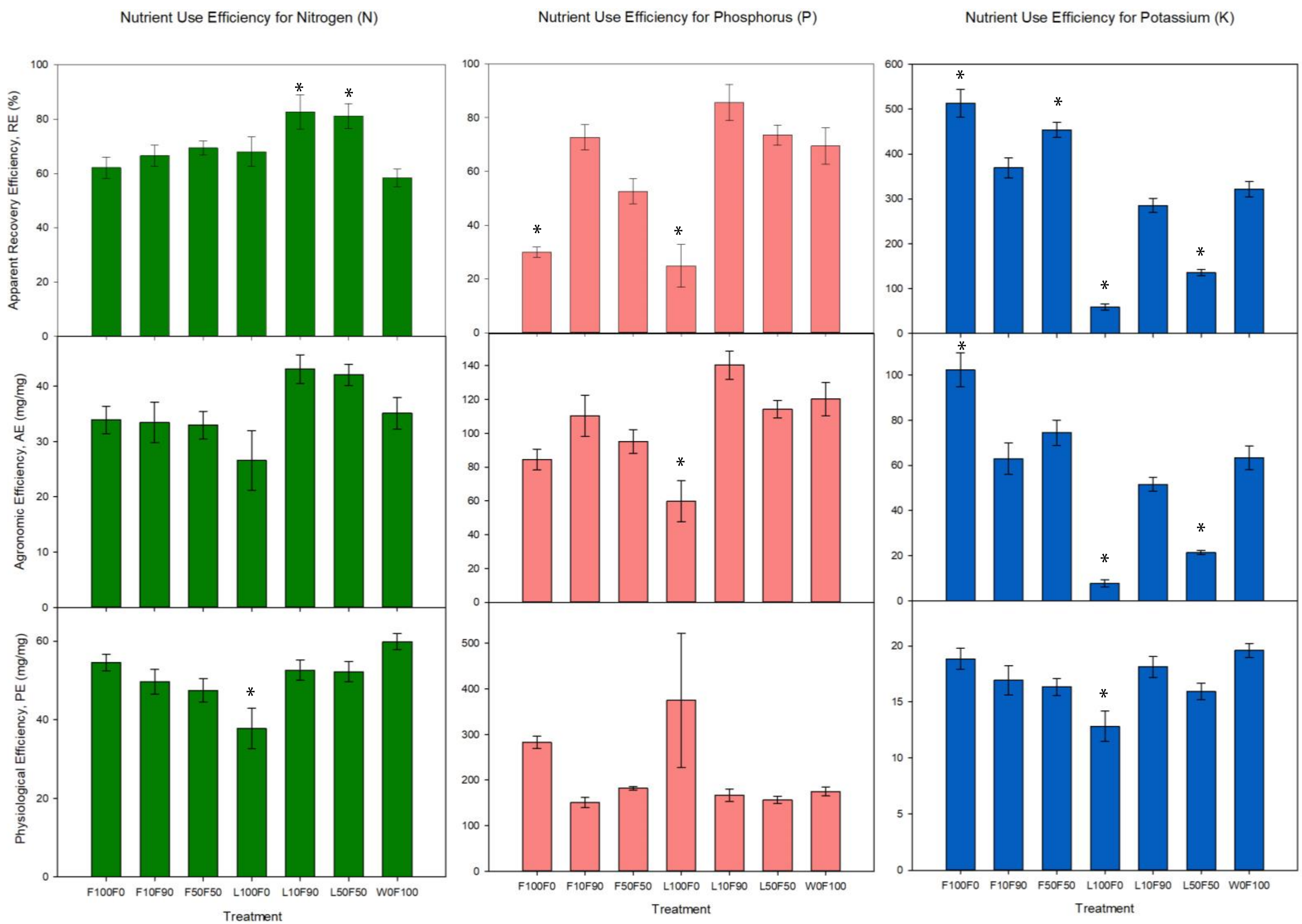


*Figure 4.11: Nutrient Use Efficiency for Nitrogen (A), Phosphorus (B) and Potassium (C). * indicates significantly different the fertilizer control.*

4.3.4 Electrical Conductivity

There was a significant effect of treatment on media EC values (Fig 4.12, p=0.001). The media from the treatments receiving pure LCD had the highest EC values (4.460 +/- 0.576 mS/cm), seconded by L50F50 (3.170 +/- 0.320). This is concerning, as an EC value of 3.2 mS/cm is considered the threshold above which yield loss begins to occur in Chinese cabbage. The lowest values were found in treatments with the smallest amounts of digestate, including F10F90, L10F90, F50F50 and the pure fertilizer treatment.

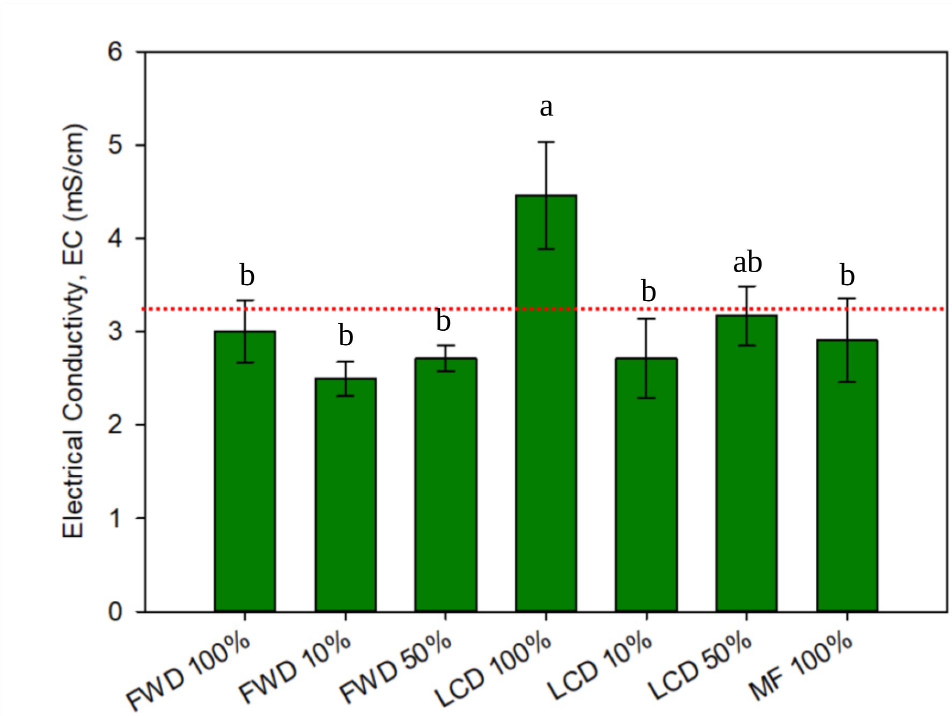


Figure 4.12: Electrical conductivity (mS/cm) by treatment. Red dotted line represents EC threshold of 3.2 mS/cm. Error bars are SE of the mean. Means that do not share a letter are significantly different.

In addition, there was a significant effect of treatment on tissue Na^+ concentration (Table 4.6, p<0.001). Plants treated with pure LCD had the highest tissue Na^+ concentrations, corresponding with the results from the EC analysis. There was also a significant negative correlation between tissue Na^+ and shoot dry weight (p=0.017, r=-0.400)

Table 4.5. Concentration of Na in plant tissue.

Treatment	Mean % Na	SE Mean	Grouping
CTRL	0.1448	0.00709	de
MF 100%	0.151	0.00691	de
FWD 10%	0.1622	0.00939	de
FWD 50%	0.1966	0.00383	cd
FWD 100%	0.1764	0.00543	de
LCD 10%	0.1692	0.00868	de
LCD 50%	0.2582	0.0144	bc
LCD 100%	0.4096	0.0346	a

Means that do not share a letter are significantly different.

4.3.5 Fecal Coliforms

All digestate samples tested below 200 most probable number (MPN) per 100mL, the detectable limit of the lab. Based on total solids of 2.87% and 1.21% for LCD and FWD respectively, this equates to less than 574 or 242 MPN per gram of total solids of LCD and FWD respectively. This is well below the EPA limit of 1,000 MPN per gram established for Class A Biosolids. Of the four treatments for which media samples were tested at the end of the growing period, only LCD 50% had a fecal coliform count significantly different from zero (22.0 ±8.35 MPN/g), indicating that digestate addition caused little to no increase of fecal coliform counts at harvest.

4.4 Discussion

4.4.1 Plant Growth and Salinity

All digestate and combined digestate and fertilizer treatments produced more biomass and longer roots than the unamended control. When compared with the fertilized control, there were small but statistically non-significant yield improvements from the combined use of LCD and fertilizer (LCD 10% and LCD 50%). Similar growth to the fertilized control was observed with pure FWD and FWD and fertilizer mixtures. Both were highest in LCD mixtures and pure fertilizer, and lowest in pure LCD treatments. No digestate or digestate-fertilizer treatments were significantly different from the pure fertilizer control for shoot DW, indicating that digestates could serve as an effective substitute for mineral fertilizers. This is similar to other research comparing plant biomass production under digestate treatments vs mineral fertilizers (Alburquerque et al, 2012; Walsh et al, 2012; Riva et al, 2016). It is important to note that in this study, the significant differences in biomass at the 100% nitrogen level were between the LCD mixtures and the pure LCD treatments, with the pure LCD treatments performing the worst. Wentzl et al (2016) found a non-linear relationship between the amount of NH_4-N applied as digestate and aboveground biomass that is similar to the findings in this study. In both cases, the benefits of application diminish and may begin to decrease with higher rates of application.

High salinity, resulting in high EC in the media treated with pure LCD likely contributed to decreased plant growth in these treatments. Plants grown in these treatments also had the highest tissue Na^+. Along with nutrients, digestates also contribute soluble Na^+ and Cl^- that can have adverse impacts on the plant root environment. This harsh environment can cause osmotic stress and ultimately lower productivity of plants. In this experiment, the LCD had a much lower nitrogen content than did the FWD, and thus more LCD digestate was needed to satisfy the nutrient requirements. Treatments that received the highest amount of LCD digestate showed the highest EC values. Along with nitrogen, digestates added more sodium and chloride, contributing to the greater observed EC in treatments with 100% and 50% of nitrogen satisfied by LCD. LCD 100% treatments had media with EC measurements above the tolerance threshold of 3.2 for Chinese cabbage (Shannon and Grieve, 1999). Beyond this threshold, yield losses are expected at a rate of 10% per unit of EC, which may explain the decline in productivity in the LCD 100% treatments. The LCD 50% treatments were just below this threshold and still produced slightly, albeit insignificantly, more biomass than did the fertilized control treatment. This is similar to findings from Tsachidou et al (2019), where substituting chemical fertilizers with biogas residues up to 65% showed no reduction in biomass and Wang et al (2019), where substituting mineral fertilizer with poultry digestate up to 50% produced beneficial results. Due to the effects of high amounts of digestate on EC, partial substitution of fertilizer with digestates having relatively low nutrient value (i.e. LCD) or partial to full substitution with digestates having relatively high nutrient value (i.e. FWD) would be recommended for *Brassica juncea*. However, Maunuksela et al (2013) found increased or similar growth of barley with digestate addition, but an inhibition of growth with Chinese cabbage. Barley is one of the most salt tolerant major crops (Shahid and Jaradat, 2013), and this highlights the fact that different plant species may be more or less tolerant to the possible negative effects of digestate addition.

4.4.2 Root Growth

Much of the research on digestates' effects on root growth centers on root biomass. There are widely varied findings from a decrease in root biomass to no difference to an increase in root biomass with digestate as compared to mineral fertilizer (Andruschkewitsch et al, 2013; Wentzel and Joergensen, 2016; Gunnarsson et al, 2010). Our investigation focused on root growth and morphology rather than root biomass in order to better understand how the effects of digestate on roots may be affecting plant growth more broadly. We found no significant differences in mean root length between treatments at the end of the growing period. This is slightly different from results by Wang et al (2019) and Zhang et al (2017) who found increased root biomass and root length when combining synthetic nutrients with digestate in hydroponic lettuce production and paddy rice production, respectively. This could be due to the difference in production systems, digestates used and/or the different ratios of nutrients to digestate utilized in the studies. The inconclusive nature of these studies point to the need for specifically understanding how different digestates function in different production systems.

Nonetheless, there were significant differences in root length in the middle of the growing period. The grouping that occurred during this seemingly critical growth period corresponded with the aboveground biomass results, with LCD 10%, LCD 50% and the fertilizer control performing best in both. This indicates that root length and/or biomass measurements taken only at the end of the growing period may not capture important differences that occur earlier in the plant life cycle, but affect yield in the longer term. Increased root growth at this early stage can be important for plant establishment and growth and may have contributed to the higher yield of these treatments (Wang et al, 2016).

Although there was only a significant difference in root hair rating due to treatment for L50F0, the highest rating was for both treatments with pure LCD in each of the respective levels of nitrogen. Root hairs increase the surface area of roots with the soil matrix and are important in plant nutrient and water acquisition. Increased root hair length and density allows plants to more effectively acquire resources. Greater root hair growth is generally associated with low nutrient conditions and can lead to improved NUE. However, in this experiment, the highest root hair length and density was not in the treatment with the lowest nutrient availability (CTRL). Instead, higher root hair ratings were observed in the treatments with pure LCD at both the 50% and 100% rate of N. While this may indicate a lack of plant-available nutrients in these treatments, in this experiment root hair length and density were not correlated with nutrient uptake. Instead, the fact that root hair growth in these treatments actually exceeded the unamended control suggests that there may be a stimulatory effect of LCD on root hair growth. Root hair growth is largely governed by plant hormones, most important of which is auxin. Previous research has found auxin and auxin-like compounds in digestates, suggesting a biostimulatory effect of digestates due to such compounds (Scaglia et al, 2015, Scaglia et al, 2017, Kostenberg et al, 1995). At low concentrations, as would be expected in digestates, auxin has a stimulatory effect on root hair and lateral root growth (Pitts et al, 1998).

4.4.3 Nutrients and Nutrient Use Efficiency

Nutrient uptake and nutrient RE are the two most common metrics for comparing digestate treatments to controls. Both of these measurements incorporate biomass yield and % of the nutrient in plant tissue, with the former being a simple measure of the total amount of the nutrient in plant biomass and the latter comparing the amount of nutrient in treated plants with uptake in untreated plants based on the total amount of that nutrient applied. AE is an additional metric that quantifies the benefit in productivity, simply calculated based on yield differences and the amount of applied fertilizer. RE and AE essentially standardize the measurement based on the amount of nutrient applied, and thus provide a good comparison between treatments that are receiving different amounts of the nutrient, as was the case with all but nitrogen in this study.

Partial substitution of fertilizer with LCD up to 50% led to a significant increase in RE and a slight but not statistically significant increase in AE for nitrogen over the pure fertilizer control. Both the %N in tissue and N uptake in W0F100 were significantly lower than in all mixtures of digestate and fertilizer for both LCD and FWD. This, combined with the slightly greater biomass in the LCD mixtures contributed to the higher RE for nitrogen in mixed LCD-fertilizer treatments. Other treatments including digestates and mixtures of digestate and fertilizers had intermediate RE values. This is different from what is currently reported in the literature, where RE with digestate was found to be similar to or lower than RE with fertilizers (de Boer, 2008; Gunnarsson, 2010; Baral et al, 2017; Tsachidou et al, 2019). Interestingly, Gunnarsson et al (2010) found lower dry mass production and NUE under digestate treatment than mineral fertilizers during early growth, and no difference at the end of a long-term 172-day study with ryegrass. This indicates that different plants may respond differently to digestate treatment during early growth stages. However, these studies were also looking at pure digestates, which in this study produced biomasses similar to or lower than pure fertilizer for both LCD and FWD. In addition, these were field-based studies in which the potential for loss of N through volatilization of NH_4 was likely higher. In this pot study, the high cation exchange capacity and low pH of the peat-based media likely limited volatilization of NH_4-N, allowing plants to utilize more of the applied N from digestates (Gunnarsson et al, 2010). The increased recovery efficiency of N in combined LCD and fertilizer treatments may have contributed to the slight increases in growth under these treatments.

Agronomic efficiency was significantly lower in the pure LCD treatments than in L10F90, L50F50, W0F100 and F10F90. This is in line with the biomass reduction likely due to the high EC of the media in this treatment. Agronomic efficiency refers to the benefit in productivity from the applied nutrient, and thus is an indicator that higher amounts of LCD reduce the benefit of the applied nitrogen, as was also observed by Wentzl et al (2016). Even though these treatments had similarly high N uptake to other treatments, and thus similar RE, the yield was low enough to significantly reduce the AE, an important metric for considering digestate use on farms.

Another important difference between digestate and fertilizers is the form of N. In synthetic fertilizers, all N is present as mineral N in the form of NH_4 or NO_3, which is readily available to plants, while in digestates, some of the N is present in organic form. In this short-term study, it is unlikely that much, if any, of the organic N present in digestates mineralized (Gunnarsson et al, 2010). However, the long-term benefits possible form the mineralization of organic N over time should not be dismissed (Möller and Müller, 2012).

Partial substitution of digestates for fertilizer did not significantly affect phosphorus RE in comparison with the pure fertilizer treatments. However, it was significantly lower in treatments with pure digestate than in treatments with fertilizer. There are differing reports on the phytoavailability of P from digestates, but most such studies compare phytoavailability of P in digested vs. undigested materials. Such studies suggest similar or higher phytoavailability with digestates compared to undigested materials and one study found that digestates increased P uptake as much as mineral fertilizer (Möller and Stinner, 2010 and Bachmann et al., 2016, respectively). However, results from this study suggest that P availability in the two digestates tested was relatively low compared with mineral fertilizer. More P was added in both pure digestate treatments than in the pure fertilizer treatment (36.8 mg per pot for FWD and 40.8 mg per pot for LCD vs. 26.8 mg per pot for fertilizer). However, a much smaller proportion of this phosphorus was actually taken up by the plants, as indicated by the lower RE in pure digestate treatments compared with mineral fertilizer treatments. Although there was a high amount of P in digestates, the pH of both FWD and LCD was high (>8), and thus may have decreased the solubility, and thus the phytoavailability, of phosphorus (Möller and Müller, 2012). Lower P availability in pure digestate treatments may also help explain the greater root hair ratings in pure LCD treatments, a plant response to low-P conditions (Jungk et al., 2001).

4.4.4 Biohazards

Fecal coliform counts for both digestates were lower than the fecal coliform limit of 1,000 MPN per gram of total solids for biosolids application to land (EPA Part 503). In order to be applied to land as Class A biosolids, materials must test below 1,000 MPN fecal coliforms or below 3 MPN *Salmonella* sp. per gram of total solids in addition to meeting one of 6 established processes to further reduce pathogens (PFRPs) that are shown to also reduce enteric viruses and viable helminth ova. In order to qualify as Class A biosolids, all digestates tested would still need to undergo additional processing, i.e. heat treatment in order to comply with regulations. However, this is a good first indicator that digestates would be suitable for land application upon further treatment.

4.4.5 Potential Implications for Sustainability

This research suggests that digestates from anaerobic digestion of both food waste and lignocellulosic materials have potential to substitute for a significant amount (≥50%) of the N fertilizer requirements for B. juncea. Bloodmeal is a common organic fertilizer that is used largely for its high content of plant-available N. In Hawaii, locally available bloodmeal has an N-content of 9.5% and cost of $0.21 per pound. Table 4.6 outlines the monetary savings that could be achieved by substituting 10% or 50% of bloodmeal N with N in the form of digestates produced from on-farm materials, assuming an already functional AD system, on various sized farms.

Table 4.6: Potential savings from replacing bloodmeal fertilizer with digestates.

Rate of replacement (%)	**1 ha farm**	**10 ha farm**	**50 ha farm**
10%	$48.44	$484.40	$2422.00
50%	$242.20	$2422.00	$12,110.00

At a target application rate of 100 kg N/ha, replacing 50% of bloodmeal N with digestates could amount to over $10,000 in savings per crop on the larger organic farms in Hawaii. However, anaerobic digestion produces two valuable products: biogas and digestate. The biogas produced would provide benefits in the form of energy, thus decreasing costs there as well. In addition to monetary savings, digestate use would contribute to overall farm sustainability by reducing the need for imported inputs. In addition, because on-farm wastes can be used as a feedstock for anaerobic digestion, this process not only reduces waste but turns it into two valuable resources. The significant potential benefits from digestate use suggest the need for further research. This research should have a specific emphasis on the effects of digestates on plant growth in Hawaii's unique agricultural soils, and include field trials with a variety of crops in order to optimize recommendations for digestate use on farms.

4.5 Conclusion

In summary, we found that partial substitution of LCD for mineral fertilizer led to small, albeit not statistically significant increases in plant biomass and that partial to full substitution with FWD produced similar biomass when compared to the fertilized control. This was associated with a significantly higher nitrogen RE and slightly, though not significantly, higher AE with LCD-fertilizer mixtures and no difference for FWD treatments. Pure LCD produced significantly lower biomass than did the other treatments at the same level of fertilization, likely due to the high EC of the media in these treatments. The agronomic efficiency was also lowest for pure LCD for N, P and K. All FWD treatments produced intermediately. Overall, these results suggest that digestates enriched in NH_4-N (i.e. FWD) can be applied at full strength or mixed with fertilizers without negatively affecting plant growth or nutrient use compared with mineral fertilizer. However, digestates lower in NH_4-N are better applied as mixtures with fertilizer for optimum benefit because of the possible negative impacts on EC due to their higher rates of application. In this case, partial substitution of fertilizer with digestate seems to offer maximum benefit for plant yield while also reducing commercial inputs. This strategy provides opportunities to reduce both costs and environmental impact while increasing farm sustainability.

Chapter 5. Conclusion

The goal of this thesis research was to better understand how digestates affect plant growth in order to optimize their use in agriculture. In pursuit of this goal, exploratory research was conducted to investigate the presence and activity of phytohormones in a nutrient-rich food waste digestate and a low-nutrient lignocellulosic digestate. Digestates were then compared to an equivalent synthetic nutrient solution to assess their effects on plant growth beyond their nutrient value alone. The effects of increasing digestate concentration on specific plant growth parameters with a focus on root growth and nutrient use efficiency was also evaluated.

In the first study, the results suggested that there may be hormone-like effects of the two digestates. Preliminary bioassays showed slight root growth stimulation at lower concentrations and root growth inhibition at higher concentrations. Initial fractionation of LCD produced one fraction that had stimulatory effects on root growth and another fraction that had inhibitory effects on root growth. Follow-up research is needed to isolate and identify the compounds responsible for these hormone-like effects. An auxin-specific bioassay was developed to investigate the auxin-like effects of digestate on an auxin-deficient *Arabidopsis* mutant. However, the mutant seeds showed unexpected growth patterns, suggesting that they may have been contaminated. If the correct mutant seeds were available, future use of this bioassay could shed light on the auxin-like effects of not only digestates, but of any biofertilizers suspected to have auxin-like effects.

In the second study, early growth of *Brassica juncea* was significantly greater under digestate treatments than their equivalent nutrient solutions. With increasing rates of digestate application, plant growth increased, while it did not increase with increasing amounts of nutrient solution, suggesting that there are beneficial effects of digestate beyond their nutrient value alone. This may be due to the effects of the organic matter in digestates and/or the activity of hormone-like compounds and warrants further study. We attributed the lack of increasing growth in the nutrient solutions to a growth-limiting effect of the nutrient solution, likely the high amounts of salt, which adversely affected plant growth. We also note that the organic constituents in the digestates may have mitigated against the salt.

In the final study, all digestates and digestate-fertilizer mixtures outperformed the unamended control. The partial substitution of mineral fertilizer with LCD led to beneficial effects on *Brassica juncea* growth. Biomass and root length were both slightly, though not significantly, higher in partial LCD treatments than in fertilizer alone. There was also increased nitrogen use efficiency with mixtures of LCD and fertilizer than fertilizer alone. There were no significant differences in biomass between the fertilizer control and any of the digestate treatments at the 100% nutrient rate, suggesting that digestates can effectively substitute or partially substitute for mineral fertilizers. However, there was elevated EC when LCD was used at full strength, along with decreased biomass (not statistically significant) and root length in this treatment.

Based on this research, LCD could partially replace mineral fertilizers for kai choy up to 50% of the target nitrogen application and may lead to increased plant growth beyond mineral fertilizers. However, full substitution leads to negative impacts on plant growth due to high EC. FWD could replace 100% of mineral fertilizer for kai choy without negatively impacting plant growth, but no beneficial effects were seen beyond the fertilizer control.

In order to develop recommendations for digestate use on farms, future research should focus on: (1) the use of digestates in different agriculturally important soils, (2) field verification of findings, (3) the effects of digestates on a variety of crops, (4) the mechanisms by which digestates affect plant growth, with a particular focus on the potential microbial and organic matter contributions of digestates and (5) an environmental and economic analysis of digestate use in Hawaiian agriculture.

This study was conducted using a peat-based media containing a high amount of organic carbon. Studies investigating digestate's effects on soils may help clarify effects that are likely due to organic matter and/or microbes added with digestate. In addition, nutrient cycling would likely be different in soil conditions as opposed to peat-based media. Field studies would be needed to verify the rates of application and effects on plant growth under more realistic growing conditions. In addition, digestate effects on plant growth with different species is likely variable. The importance of salinity tolerance in plant response to digestates was shown in this research, suggesting that further investigation on the effects of digestate with different crops would help inform its use in a variety of cropping systems. The mechanisms by which digestates affect plant growth are still largely unknown. In addition to mineral nutrients, digestates contain both microorganisms and organic compounds, both of which could be contributing to their beneficial effects on plant growth through a variety of mechanisms. A better understanding of how the biotic components of digestates interact with both soil and plants will help inform their use in agriculture. Lastly, a comprehensive economic and environmental analysis of the potential for digestate would provide additional information both to encourage its use on farm and help inform policy regarding its regulation.

Appendices

Appendix 2.1: Biogas production during food waste digestion. (*indicates missing data)

DATE	Day	BAG	Cum Vol (L)	% Methane
1-Aug	2	1FWD	2.042718564	4.04
1-Aug	2	2FWD	2.171422704	6.041
1-Aug	2	3FWD	2.254161079	8.138
1-Aug	2	4FWD	2.241290665	5.327
1-Aug	2	5FWD	2.364478914	5.702
1-Aug	2	6FWD	2.25599971	6.479
1-Aug	2	1DIG	0.347501178	19.673
1-Aug	2	2DIG	0.312567197	20.252
1-Aug	2	3DIG	0.365887484	23.835
9-Aug	10	1FWD	4.265043331	50.272
9-Aug	10	2FWD	4.3019917	57.398
9-Aug	10	3FWD	4.478320962	54.909
9-Aug	10	4FWD	4.582897934	49.05
9-Aug	10	5FWD	4.528079987	55.392
9-Aug	10	6FWD	4.362712206	56.755
9-Aug	10	1DIG	0.942078572	*
9-Aug	10	2DIG	0.886958322	49.139
9-Aug	10	3DIG	0.962299993	56.198
14-Aug	15	1FWD	6.122180131	73.772
14-Aug	15	2FWD	7.017962514	76.796
14-Aug	15	3FWD	7.260355931	75.017
14-Aug	15	4FWD	6.913494511	73.968
14-Aug	15	5FWD	6.95226745	75.452
14-Aug	15	6FWD	6.737351553	76.331
14-Aug	15	1DIG	1.213675654	51.847
14-Aug	15	2DIG	1.094326364	56.356
14-Aug	15	3DIG	1.140306189	67.154
20-Aug	21	1FWD	8.838814828	*
20-Aug	21	2FWD	9.559864444	79.526
20-Aug	21	3FWD	10.01929435	78.771
20-Aug	21	4FWD	9.070984355	77.771
20-Aug	21	5FWD	9.067453572	78.05
20-Aug	21	6FWD	8.931627242	79.418
20-Aug	21	1DIG	1.366336913	48.356
20-Aug	2	2DIG	1.254344792	50.337
20-Aug	21	3DIG	1.326074709	69.078
29-Aug	30	1FWD	11.68651784	79.358
29-Aug	30	2FWD	11.48017228	81.752
29-Aug	30	3FWD	12.15368744	*
29-Aug	30	4FWD	11.6261657	*

DATE	Day	BAG	Cum Vol (L)	% Methane
29-Aug	30	6FWD	11.93343473	*
29-Aug	30	1DIG	1.538897011	39.68
29-Aug	30	2DIG	1.395530327	47.421
29-Aug	30	3DIG	1.495866463	50.413
4-Sep	36	1FWD	16.26082217	57.099
4-Sep	36	2FWD	16.44600838	61.369
4-Sep	36	3FWD	16.63148746	*
4-Sep	36	4FWD	16.74181326	60.631
4-Sep	36	5FWD	16.10003577	58.551
4-Sep	36	6FWD	17.15753475	60.126
4-Sep	36	1DIG	1.614262282	49.208
4-Sep	36	2DIG	1.466300155	47.842
4-Sep	36	3DIG	1.573989	*
12-Sep	44	1FWD	17.85452193	81.47
12-Sep	44	2FWD	17.93860839	80.709
12-Sep	44	3FWD	18.00276776	78.195
12-Sep	44	4FWD	17.94949578	56.454
12-Sep	44	5FWD	17.4363907	79.581
12-Sep	44	6FWD	18.5251387	79.928
12-Sep	44	1DIG	1.704332972	22.756
12-Sep	44	2DIG	1.526960007	45.902
12-Sep	44	3DIG	1.653030626	*
19-Sep	51	1FWD	22.99709597	58.854
19-Sep	51	2FWD	23.05015359	57.248
19-Sep	51	3FWD	23.40726172	59.897
19-Sep	51	4FWD	22.90315894	55.234
19-Sep	51	5FWD	22.37727728	54.839
19-Sep	51	6FWD	23.73615871	59.289
19-Sep	51	1DIG	1.757082002	*
19-Sep	51	2DIG	1.547037492	*
19-Sep	51	3DIG	1.682234241	*
1-Oct	63	1FWD	24.97217632	82.014
1-Oct	63	2FWD	24.66794208	81.397
1-Oct	63	3FWD	24.95613097	*
1-Oct	63	4FWD	25.03421441	76.466
1-Oct	63	5FWD	24.65705321	*
1-Oct	63	6FWD	25.18527643	80.961
1-Oct	63	1DIG	1.822373914	20.364
1-Oct	63	2DIG	1.589658602	36.912
1-Oct	63	3DIG	1.75024665	13.641

Appendix 2.2: Root length data for initial cucumber bioassay.

Block	CONC (%)	DIG	mm
A	0	ctrl	15.32685
A	0	ctrl	10.231892
A	0	ctrl	11.679882
A	0	ctrl	16.282743
A	0	ctrl	18.712456
A	0	ctrl	15.947952
A	0	ctrl	10.274404
A	0	ctrl	16.059002
A	0	ctrl	11.697464
A	0	ctrl	20.035467
A	0	ctrl	15.659419
A	0	ctrl	15.763994
A	0	ctrl	12.132787
A	0	ctrl	15.651746
A	0	ctrl	17.405376
B	0	ctrl	13.3892
B	0	ctrl	15.092359
B	0	ctrl	22.557187
B	0	ctrl	20.351274
B	0	ctrl	16.724052
B	0	ctrl	17.672911
B	0	ctrl	17.320464
B	0	ctrl	15.24525
B	0	ctrl	19.394668
B	0	ctrl	19.512843
B	0	ctrl	14.691348
B	0	ctrl	14.69714
B	0	ctrl	17.730447
B	0	ctrl	20.116472
B	0	ctrl	16.720444
C	0	ctrl	14.659703
C	0	ctrl	19.431214
C	0	ctrl	14.376472
C	0	ctrl	15.048561
C	0	ctrl	13.925761
C	0	ctrl	12.441999
C	0	ctrl	17.511749
C	0	ctrl	16.967978
C	0	ctrl	14.322715
C	0	ctrl	14.824413
C	0	ctrl	14.668705
C	0	ctrl	13.539356
C	0	ctrl	13.744184
C	0	ctrl	16.353168
A	0.1	FWD	15.803927
A	0.1	FWD	15.737246
A	0.1	FWD	16.957866
A	0.1	FWD	15.608715
A	0.1	FWD	14.281509
A	0.1	FWD	21.06875
A	0.1	FWD	8.394652
A	0.1	FWD	14.756418
A	0.1	FWD	17.381393
A	0.1	FWD	18.812307
A	0.1	FWD	17.503737
A	0.1	FWD	16.279567
A	0.1	FWD	11.518654
A	0.1	FWD	19.724152
B	0.1	FWD	16.860133
B	0.1	FWD	10.800172
B	0.1	FWD	18.646328
B	0.1	FWD	13.965803
B	0.1	FWD	15.065781
B	0.1	FWD	13.969
B	0.1	FWD	12.901492
B	0.1	FWD	14.575646
B	0.1	FWD	14.30942
B	0.1	FWD	12.696252
B	0.1	FWD	11.059443
B	0.1	FWD	14.126773
B	0.1	FWD	9.786018
C	0.1	FWD	19.800539
C	0.1	FWD	9.207679
C	0.1	FWD	15.806121
C	0.1	FWD	18.341317
C	0.1	FWD	17.466136
C	0.1	FWD	13.322983
C	0.1	FWD	13.02536
C	0.1	FWD	16.637198
C	0.1	FWD	18.613992
C	0.1	FWD	20.406103
C	0.1	FWD	15.893967
C	0.1	FWD	17.765867
C	0.1	FWD	18.85885
A	1	FWD	15.538292
A	1	FWD	17.615322
A	1	FWD	16.706005
A	1	FWD	13.961555
A	1	FWD	22.527933
A	1	FWD	18.059995
A	1	FWD	15.94129
A	1	FWD	19.262664
A	1	FWD	14.071823
A	1	FWD	15.811695
A	1	FWD	21.253648
A	1	FWD	9.7590065
A	1	FWD	15.622982
B	1	FWD	15.043286
B	1	FWD	16.650509
B	1	FWD	16.353585
B	1	FWD	12.860106
B	1	FWD	14.458383
B	1	FWD	17.080563
B	1	FWD	17.656147
B	1	FWD	19.216272
B	1	FWD	13.751996
B	1	FWD	17.483938
B	1	FWD	12.009068
B	1	FWD	18.635074
B	1	FWD	19.452788
B	1	FWD	25.764956
B	1	FWD	16.940467
C	1	FWD	16.282632
C	1	FWD	17.4502
C	1	FWD	6.918445
C	1	FWD	10.744188
C	1	FWD	12.943726
C	1	FWD	23.308585
C	1	FWD	22.981715
C	1	FWD	12.898784

Block	CONC (%)	DIG	mm
C	1	FWD	16.547438
C	1	FWD	17.637817
C	1	FWD	18.183414
C	1	FWD	9.226486
C	1	FWD	17.592226
C	1	FWD	20.838594
A	5	FWD	13.746217
A	5	FWD	18.450531
A	5	FWD	13.411679
A	5	FWD	12.444974
A	5	FWD	14.499656
A	5	FWD	13.76296
A	5	FWD	16.611143
A	5	FWD	16.25959
A	5	FWD	18.595382
A	5	FWD	16.356997
A	5	FWD	16.058306
A	5	FWD	12.395923
B	5	FWD	14.525921
B	5	FWD	14.999404
B	5	FWD	17.768078
B	5	FWD	19.871536
B	5	FWD	14.834226
B	5	FWD	20.451121
B	5	FWD	16.673489
B	5	FWD	17.005825
B	5	FWD	16.142412
B	5	FWD	20.680616
B	5	FWD	17.431048
B	5	FWD	17.65584
B	5	FWD	18.769763
B	5	FWD	14.070752
C	5	FWD	18.36904
C	5	FWD	14.639827
C	5	FWD	12.869617
C	5	FWD	20.271584
C	5	FWD	17.924627
C	5	FWD	17.747915
C	5	FWD	13.971042
C	5	FWD	16.615583
C	5	FWD	22.147226
C	5	FWD	20.980074
C	5	FWD	16.571974
C	5	FWD	17.415438
C	5	FWD	15.169406
C	5	FWD	17.745344
C	5	FWD	8.258882
A	10	FWD	13.376589
A	10	FWD	11.85117
A	10	FWD	18.705714
A	10	FWD	14.187471
A	10	FWD	15.037285
A	10	FWD	22.092793
A	10	FWD	13.398169
A	10	FWD	12.437799
A	10	FWD	12.838337
A	10	FWD	13.14716
A	10	FWD	16.670121
A	10	FWD	19.575104
A	10	FWD	12.634689
A	10	FWD	18.125646
A	10	FWD	17.090725

Block	CONC (%)	DIG	mm
B	10	FWD	11.63831
B	10	FWD	10.678836
B	10	FWD	11.801069
B	10	FWD	14.053664
B	10	FWD	20.158453
B	10	FWD	18.000475
B	10	FWD	13.369457
B	10	FWD	19.878838
B	10	FWD	9.854852
B	10	FWD	14.3635
B	10	FWD	16.419677
B	10	FWD	12.47113
B	10	FWD	19.626411
C	10	FWD	16.812989
C	10	FWD	15.760506
C	10	FWD	19.812992
C	10	FWD	20.564215
C	10	FWD	16.654003
C	10	FWD	15.820262
C	10	FWD	16.123215
C	10	FWD	12.902068
C	10	FWD	16.520585
C	10	FWD	16.617463
C	10	FWD	22.493663
C	10	FWD	17.776849
C	10	FWD	16.544749
C	10	FWD	19.337258
A	20	FWD	15.57645
A	20	FWD	15.582216
A	20	FWD	13.447924
A	20	FWD	11.987236
A	20	FWD	6.4611125
A	20	FWD	8.317884
A	20	FWD	14.215269
A	20	FWD	16.694552
A	20	FWD	17.042575
A	20	FWD	16.137818
A	20	FWD	10.378522
A	20	FWD	16.596185
A	20	FWD	15.756058
A	20	FWD	12.550203
A	20	FWD	19.018074
B	20	FWD	10.020031
B	20	FWD	15.903854
B	20	FWD	15.711155
B	20	FWD	12.457207
B	20	FWD	10.020883
B	20	FWD	15.507164
B	20	FWD	9.1507405
B	20	FWD	18.704036
B	20	FWD	14.864637
B	20	FWD	14.210199
B	20	FWD	13.275588
B	20	FWD	16.366941
B	20	FWD	10.730107
B	20	FWD	17.920477
C	20	FWD	14.683017
C	20	FWD	11.895164
C	20	FWD	11.159192
C	20	FWD	14.087728
C	20	FWD	19.013314
C	20	FWD	15.324076

Block	CONC (%)	DIG	mm
C	20	FWD	15.5451
C	20	FWD	17.958723
C	20	FWD	14.732112
C	20	FWD	18.604878
C	20	FWD	14.717708
C	20	FWD	13.634211
C	20	FWD	13.55574
C	20	FWD	16.40019
A	50	FWD	6.9877934
A	50	FWD	10.239611
A	50	FWD	10.545628
A	50	FWD	13.720518
A	50	FWD	10.120369
A	50	FWD	8.429629
A	50	FWD	15.758517
A	50	FWD	14.743785
A	50	FWD	11.636541
A	50	FWD	7.308779
A	50	FWD	10.431225
A	50	FWD	13.277775
A	50	FWD	13.092182
A	50	FWD	10.320258
B	50	FWD	11.466501
B	50	FWD	12.509668
B	50	FWD	10.911143
B	50	FWD	12.131584
B	50	FWD	5.898295
B	50	FWD	9.383304
B	50	FWD	11.40288
B	50	FWD	10.115868
B	50	FWD	11.082587
B	50	FWD	13.214098
B	50	FWD	8.4808123
B	50	FWD	13.382785
B	50	FWD	13.695481
B	50	FWD	9.4409734
C	50	FWD	14.498044
C	50	FWD	13.236984
C	50	FWD	12.60079
C	50	FWD	13.720212
C	50	FWD	11.393671
C	50	FWD	11.541498
C	50	FWD	9.5308506
C	50	FWD	9.8919773
C	50	FWD	16.163447
C	50	FWD	17.350882
C	50	FWD	15.679644
C	50	FWD	15.313555
C	50	FWD	14.454167
A	100	FWD	9.2070836
A	100	FWD	6.162252
A	100	FWD	6.0557663
A	100	FWD	8.8039523
A	100	FWD	6.787494
A	100	FWD	8.9735943
A	100	FWD	9.709673
A	100	FWD	5.762491
A	100	FWD	5.669621
A	100	FWD	7.158209
A	100	FWD	5.7498485
A	100	FWD	6.297847
A	100	FWD	7.381684

Block	CONC (%)	DIG	mm
B	100	FWD	8.386318
B	100	FWD	8.1879586
B	100	FWD	5.7768816
B	100	FWD	6.945074
B	100	FWD	8.283054
B	100	FWD	7.502244
B	100	FWD	6.521978
B	100	FWD	5.476466
B	100	FWD	5.660071
B	100	FWD	7.362585
C	100	FWD	9.501313
C	100	FWD	5.3621
C	100	FWD	5.437493
C	100	FWD	6.51487
C	100	FWD	6.289856
C	100	FWD	6.528309
C	100	FWD	5.73007
C	100	FWD	7.183298
C	100	FWD	8.143066
C	100	FWD	6.3660985
A	0.1	LCD	16.54347
A	0.1	LCD	15.609093
A	0.1	LCD	11.383703
A	0.1	LCD	13.723874
A	0.1	LCD	12.788048
A	0.1	LCD	16.615838
A	0.1	LCD	16.504813
A	0.1	LCD	18.583189
A	0.1	LCD	15.255193
A	0.1	LCD	15.732013
A	0.1	LCD	13.209661
A	0.1	LCD	14.307342
A	0.1	LCD	16.514308
A	0.1	LCD	20.563912
A	0.1	LCD	13.207682
B	0.1	LCD	14.607121
B	0.1	LCD	14.176571
B	0.1	LCD	13.162886
B	0.1	LCD	10.440729
B	0.1	LCD	18.245754
B	0.1	LCD	17.713512
B	0.1	LCD	18.294855
B	0.1	LCD	14.242605
B	0.1	LCD	17.095997
B	0.1	LCD	15.56152
B	0.1	LCD	16.297221
B	0.1	LCD	11.063087
B	0.1	LCD	17.019151
B	0.1	LCD	17.934828
B	0.1	LCD	14.739567
C	0.1	LCD	17.041132
C	0.1	LCD	19.2509
C	0.1	LCD	14.661969
C	0.1	LCD	15.404857
C	0.1	LCD	18.366599
C	0.1	LCD	14.791687
C	0.1	LCD	17.813115
C	0.1	LCD	17.855206
C	0.1	LCD	18.940697
C	0.1	LCD	22.419095
C	0.1	LCD	17.815778
C	0.1	LCD	8.845667

Block	CONC (%)	DIG	mm
C	0.1	LCD	16.028174
A	1	LCD	19.321434
A	1	LCD	17.53437
A	1	LCD	16.502497
A	1	LCD	16.529945
A	1	LCD	21.19728
A	1	LCD	19.31627
A	1	LCD	21.969566
A	1	LCD	11.83215
A	1	LCD	17.786976
A	1	LCD	14.226377
A	1	LCD	20.788882
A	1	LCD	19.684502
A	1	LCD	16.99299
A	1	LCD	18.428178
B	1	LCD	21.0048
B	1	LCD	21.72041
B	1	LCD	14.638891
B	1	LCD	9.9199
B	1	LCD	17.280711
B	1	LCD	11.806153
B	1	LCD	11.541731
B	1	LCD	15.09209
B	1	LCD	18.496872
B	1	LCD	13.629731
B	1	LCD	17.732626
B	1	LCD	15.700225
B	1	LCD	19.259462
B	1	LCD	15.796192
B	1	LCD	19.437777
C	1	LCD	12.909616
C	1	LCD	17.010748
C	1	LCD	21.62307
C	1	LCD	16.35536
C	1	LCD	11.689239
C	1	LCD	16.17589
C	1	LCD	16.06922
C	1	LCD	21.970236
C	1	LCD	20.72216
C	1	LCD	18.779755
C	1	LCD	17.045882
C	1	LCD	19.577414
C	1	LCD	15.913126
C	1	LCD	18.533413
C	1	LCD	19.72394
A	5	LCD	13.46808
A	5	LCD	15.969337
A	5	LCD	16.569465
A	5	LCD	13.469677
A	5	LCD	13.73184
A	5	LCD	18.5488
A	5	LCD	13.104148
A	5	LCD	18.789886
A	5	LCD	15.773395
A	5	LCD	17.191405
A	5	LCD	12.038273
A	5	LCD	16.505511
A	5	LCD	16.460968
A	5	LCD	14.132674
A	5	LCD	18.110228
B	5	LCD	12.998853
B	5	LCD	11.456467

Block	CONC (%)	DIG	mm
B	5	LCD	19.901751
B	5	LCD	18.611453
B	5	LCD	20.069726
B	5	LCD	21.04646
B	5	LCD	19.075848
B	5	LCD	13.179073
B	5	LCD	14.289666
B	5	LCD	17.080534
B	5	LCD	23.559134
B	5	LCD	14.607681
B	5	LCD	11.922044
B	5	LCD	15.035344
A	10	LCD	12.31556
A	10	LCD	16.307272
A	10	LCD	15.343798
A	10	LCD	20.672777
A	10	LCD	14.463099
A	10	LCD	12.579463
A	10	LCD	16.561996
A	10	LCD	14.266592
A	10	LCD	18.995304
A	10	LCD	17.174821
A	10	LCD	12.68793
A	10	LCD	20.199068
A	10	LCD	12.363578
A	10	LCD	17.318603
A	10	LCD	10.587002
B	10	LCD	15.763526
B	10	LCD	9.8090595
B	10	LCD	14.581368
B	10	LCD	19.230279
B	10	LCD	16.702749
B	10	LCD	19.018778
B	10	LCD	18.915054
B	10	LCD	15.754988
B	10	LCD	16.975372
B	10	LCD	13.882276
B	10	LCD	11.230183
B	10	LCD	9.421266
B	10	LCD	14.44344
B	10	LCD	16.772109
B	10	LCD	14.948791
C	10	LCD	20.995624
C	10	LCD	18.670992
C	10	LCD	15.209118
C	10	LCD	14.902802
C	10	LCD	22.549913
C	10	LCD	21.144776
C	10	LCD	16.987275
C	10	LCD	19.65543
C	10	LCD	19.658332
C	10	LCD	14.508363
C	10	LCD	20.453148
C	10	LCD	15.523307
C	10	LCD	18.198982
C	10	LCD	14.806646
A	20	LCD	15.07019
A	20	LCD	16.158147
A	20	LCD	13.190213
A	20	LCD	12.801535
A	20	LCD	6.2003475
A	20	LCD	15.488992

Block	CONC (%)	DIG	mm
A	20	LCD	9.0839726
A	20	LCD	14.126322
A	20	LCD	17.228152
A	20	LCD	17.094792
A	20	LCD	9.749159
A	20	LCD	16.443287
A	20	LCD	13.155185
B	20	LCD	13.591589
B	20	LCD	12.145245
B	20	LCD	9.7626394
B	20	LCD	16.391251
B	20	LCD	12.189941
B	20	LCD	9.479869
B	20	LCD	13.027735
B	20	LCD	14.084466
B	20	LCD	14.005004
B	20	LCD	11.713728
B	20	LCD	14.408846
B	20	LCD	13.477337
B	20	LCD	12.359604
B	20	LCD	10.380253
B	20	LCD	18.887166
A	50	LCD	11.464397
A	50	LCD	12.440733
A	50	LCD	14.21836
A	50	LCD	12.542295
A	50	LCD	7.826169
A	50	LCD	10.798286
A	50	LCD	11.865525
A	50	LCD	11.6624
A	50	LCD	9.626542
A	50	LCD	10.042152
A	50	LCD	10.514293
A	50	LCD	11.144526
A	50	LCD	8.237434
B	50	LCD	10.321759
B	50	LCD	11.670406
B	50	LCD	13.572803
B	50	LCD	13.177489
B	50	LCD	13.008395
B	50	LCD	10.615567
B	50	LCD	7.998544
B	50	LCD	11.004509
B	50	LCD	14.532706
B	50	LCD	10.719823
B	50	LCD	13.17511
B	50	LCD	12.622919
B	50	LCD	17.217315
B	50	LCD	14.874743
B	50	LCD	13.932768
C	50	LCD	13.250227
C	50	LCD	11.309667

Block	CONC (%)	DIG	mm
C	50	LCD	9.7664976
C	50	LCD	12.530856
C	50	LCD	13.977433
C	50	LCD	11.595871
C	50	LCD	12.260847
C	50	LCD	14.41753
C	50	LCD	11.724981
C	50	LCD	10.733693
C	50	LCD	16.109936
C	50	LCD	12.247405
C	50	LCD	14.283701
C	50	LCD	11.748304
A	100	LCD	9.3604255
A	100	LCD	10.649155
A	100	LCD	9.6421236
A	100	LCD	8.3054674
A	100	LCD	11.531289
A	100	LCD	8.8309956
A	100	LCD	12.740123
A	100	LCD	10.634625
A	100	LCD	8.725497
A	100	LCD	10.535235
A	100	LCD	13.345364
A	100	LCD	9.5829487
A	100	LCD	11.64489
A	100	LCD	10.38044
B	100	LCD	7.176362
B	100	LCD	11.351544
B	100	LCD	10.416138
B	100	LCD	9.09783
B	100	LCD	12.289418
B	100	LCD	11.841133
B	100	LCD	8.259337
B	100	LCD	9.532276
B	100	LCD	10.921688
B	100	LCD	12.820451
B	100	LCD	11.587573
B	100	LCD	10.304745
B	100	LCD	9.3255955
C	100	LCD	6.955477
C	100	LCD	9.637578
C	100	LCD	10.151787
C	100	LCD	9.381279
C	100	LCD	9.424255
C	100	LCD	11.290275
C	100	LCD	10.541312
C	100	LCD	6.766865
C	100	LCD	12.561122
C	100	LCD	10.120658
C	100	LCD	11.85367
C	100	LCD	10.58793
C	100	LCD	10.381917

Appendix 2.3: Root length data after fractionation.

Fraction	Length (mm)
1	35
1	41
1	28
1	36
1	34
1	39
1	36
1	39
1	26
2	32
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20	25
20	40
20	41
21	34
21	39
21	34
21	38
21	31
21	19
21	35
21	37
21	30
21	30
22	33
22	43
22	48
22	17
22	45
22	39
22	44
22	45
22	40
22	35

Appendix 2.4: Root length data for auxin-specific bioassay.

Replicate	Treatment	Seed type	Length (mm)
1	DIW	WT	20.192
1	DIW	WT	7.551
1	DIW	WT	19.558
1	DIW	WT	4.463
1	DIW	WT	9.303
1	DIW	WT	9.177
1	DIW	WT	8.039
1	DIW	WT	6.113
1	DIW	WT	7.828
1	DIW	WT	8.783
1	DIW	YQ	9.707
1	DIW	YQ	0.832
1	DIW	YQ	7.464
1	DIW	YQ	22.997
1	DIW	YQ	22.387
1	DIW	YQ	27.151
1	DIW	YQ	26.681
1	DIW	YQ	17.886
2	DIW	WT	9.737
2	DIW	WT	9.377
2	DIW	WT	9.455
2	DIW	WT	9.195
2	DIW	WT	8.125
2	DIW	WT	9.618
2	DIW	WT	8.217
2	DIW	WT	7.73
2	DIW	WT	7.575
2	DIW	WT	8.454
2	DIW	YQ	7.674
2	DIW	YQ	22.554
2	DIW	YQ	23.956
2	DIW	YQ	6.958
2	DIW	YQ	5.727
2	DIW	YQ	7.343
2	DIW	YQ	8.714
2	DIW	YQ	10.825
3	DIW	WT	7.906
3	DIW	WT	10.7
3	DIW	WT	13.155
3	DIW	WT	10.364
3	DIW	WT	7.82
3	DIW	WT	9.026
3	DIW	WT	8.855
3	DIW	WT	8.169
3	DIW	WT	9.02
3	DIW	YQ	9.7
3	DIW	YQ	13.996
3	DIW	YQ	16.887
3	DIW	YQ	7.639
3	DIW	YQ	19.039
3	DIW	YQ	16.896
4	DIW	WT	7.029

Replicate	Treatment	Seed type	Length (mm)
4	DIW	WT	8.072
4	DIW	WT	9.048
4	DIW	WT	8.49
4	DIW	WT	8.844
4	DIW	WT	8.828
4	DIW	WT	8.707
4	DIW	WT	9.692
4	DIW	WT	9.87
4	DIW	YQ	17.887
4	DIW	YQ	16.857
4	DIW	YQ	20.564
4	DIW	YQ	18.194
4	DIW	YQ	6.981
4	DIW	YQ	6.116
4	DIW	YQ	5.871
4	DIW	YQ	25.279
4	DIW	YQ	22.473
5	DIW	WT	8.209
5	DIW	WT	8.007
5	DIW	WT	8.123
5	DIW	WT	7.572
5	DIW	WT	7.57
5	DIW	WT	7.442
5	DIW	WT	9.156
5	DIW	WT	9.125
5	DIW	WT	9.057
5	DIW	WT	6.916
5	DIW	YQ	6.601
5	DIW	YQ	16.324
5	DIW	YQ	20.185
5	DIW	YQ	9.779
5	DIW	YQ	21.345
5	DIW	YQ	12.583
5	DIW	YQ	7.754
6	DIW	WT	9.255
6	DIW	WT	7.484
6	DIW	WT	8.373
6	DIW	WT	8.038
6	DIW	WT	6.507
6	DIW	WT	7.586
6	DIW	WT	9.265
6	DIW	WT	7.69
6	DIW	WT	9.218
6	DIW	WT	8.714
6	DIW	YQ	6.416
6	DIW	YQ	21.847
6	DIW	YQ	17.939
6	DIW	YQ	7.668
6	DIW	YQ	23.866
6	DIW	YQ	6.973
6	DIW	YQ	9.198

Appendix 3.1: Plant dry weight by treatment

Block	Treatment	Dry Weight (g)
1	FD-1	0.148
1	FD-5	0.611
1	FD-6	0.966
1	FN-1	0.017
1	FN-3	0.253
1	FN-5	0.188
1	FN-6	0.104
1	LD-1	0.206
1	LD-3	0.276
1	LD-5	0.842
1	LD-6	0.429
1	LN-1	0.177
1	LN-3	0.236
1	LN-5	0.098
1	LN-6	0.105
1	MG	1.052
2	CTRL	0.146
2	FD-1	0.022
2	FD-3	0.033
2	FD-5	0.78
2	FD-6	0.965
2	FN-1	0.192
2	FN-3	0.265
2	FN-5	0.047
2	FN-6	0.187
2	LD-1	0.063
2	LD-3	0.403
2	LD-5	0.386
2	LD-6	0.514
2	LN-1	0.081
2	LN-3	0.332
2	LN-5	0.47
2	MG	0.151
3	CTRL	0.034
Block	**Treatment**	**Dry Weight (g)**
3	FD-5	0.703
3	FD-6	0.845
3	FN-1	0.358
3	FN-3	0.222
3	FN-5	0.279
3	FN-6	0.129
3	LD-1	0.033
3	LD-3	0.026
3	LD-5	0.556
3	LD-6	0.7
3	LN-1	0.387
3	LN-3	0.066
3	LN-5	0.112
3	MG	1.245
4	CTRL	0.234
4	FD-1	0.243
4	FD-3	0.415
4	FD-5	0.63
4	FD-6	0.594
4	FN-1	0.564
4	FN-3	0.297
4	FN-5	0.096
4	FN-6	0.377
4	LD-1	0.055
4	LD-3	0.512
4	LD-5	0.673
4	LD-6	0.682
4	LN-1	0.074
4	LN-3	0.182
4	LN-5	0.567
4	LN-6	0.058
4	MG	0.479

Appendix 4.1: Shoot fresh and dry weight.

Blk	Treatment	Shoot FW	Shoot DW
1	W0F0	10.5	1.25
1	W0F50	13.5	1.469
1	W0F100	52.9	5.423
1	L10F40	17.4	1.749
1	L10F90	64	5.555
1	L50F0	13	0.901
1	L50F50	50.3	4.833
1	L100F0	54.9	5.114
1	F10F40	23.2	2.685
1	F10F90	37.1	3.828
1	F50F0	21.6	2.287
1	F50F50	40	3.515
1	F100F0	46.8	4.987
2	W0F0	9.4	1.109
2	W0F50	18.6	2.102
2	W0F100	43.3	4.515
2	L10F40	28.5	2.816
2	L10F90	54.3	5.498
2	L50F0	23.3	2.021
2	L50F50	51.9	5.217
2	L100F0	47.6	4.649
2	F10F40	31.2	3.435
2	F10F90	46.1	4.712
2	F50F0	24.7	2.798
2	F50F50	48	4.511
2	F100F0	38.7	3.887
3	W0F0	9.9	1.192
3	W0F50	27.7	2.834
3	W0F100	45.3	4.577
3	L10F40	31.3	3.673
3	L10F90	46.2	4.35
3	L50F0	31.5	2.544

Blk	Treatment	Shoot FW	Shoot DW
3	L100F0	35.2	3.373
3	F10F40	31.1	3.450
3	F10F90	49.6	4.741
3	F50F0	25	2.884
3	F50F50	41.6	4.12
3	F100F0	49.8	4.797
4	W0F0	10.1	1.271
4	W0F50	21.1	2.261
4	W0F100	37.4	4.021
4	L10F40	27.5	3.216
4	L10F90	45	5.145
4	L50F0	32.5	3.515
4	L50F50	46	4.668
4	L100F0	33.6	2.74
4	F10F40	22.2	2.481
4	F10F90	47.2	5.16
4	F50F0	29.2	3.231
4	F50F50	46	4.79
4	F100F0	36	3.874
5	W0F0	12.2	1.598
5	W0F50	11.7	2.15
5	W0F100	37.2	3.972
5	L10F40	22.3	2.616
5	L10F90	55.5	5.632
5	L50F0	25.9	2.429
5	L50F50	55.4	5.63
5	L100F0	33.9	2.726
5	F10F40	27	3.032
5	F10F90	35.3	3.312
5	F50F0	25.4	3.033
5	F50F50	43.5	4.596
5	F100F0	38.7	4.414

Appendix 4.2: Root length data

Block	Treatment	DAY	Intersects	Length (mm)
A	F10F40	18	78	61.261005
B	F10F40	18	51	40.0552725
C	F10F40	18	38	29.845105
D	F10F40	18	66	51.836235
E	F10F40	18	39	30.6305025
A	F10F90	18	53	41.6260675
B	F10F90	18	73	57.3340175
C	F10F90	18	65	51.0508375
D	F10F90	18	63	49.4800425
E	F10F90	18	22	17.278745
A	F50F0	18	38	29.845105
B	F50F0	18	20	15.70795
C	F50F0	18	25	19.6349375
D	F50F0	18	17	13.3517575
E	F50F0	18	34	26.703515
A	F50F50	18	23	18.0641425
B	F50F50	18	26	20.420335
C	F50F50	18	38	29.845105
D	F50F50	18	79	62.0464025
E	F50F50	18	62	48.694645
A	F100F0	18	32	25.13272
B	F100F0	18	13	10.2101675
D	F100F0	18	35	27.4889125
E	F100F0	18	36	28.27431
A	L10F40	18	12	9.42477
B	L10F40	18	68	53.40703
C	L10F40	18	97	76.1835575
D	L10F40	18	70	54.977825
E	L10F40	18	46	36.128285
A	L10F90	18	52	40.84067
B	L10F90	18	87	68.3295825
C	L10F90	18	53	41.6260675
D	L10F90	18	39	30.6305025
E	L10F90	18	66	51.836235
A	L50F0	18	0	0
B	L50F0	18	8	6.28318
C	L50F0	18	24	18.84954
D	L50F0	18	35	27.4889125
E	L50F0	18	30	23.561925
A	L50F50	18	45	35.3428875
B	L50F50	18	67	52.6216325
C	L50F50	18	50	39.269875
D	L50F50	18	32	25.13272
E	L50F50	18	84	65.97339
B	L100F0	18	19	14.9225525
C	L100F0	18	30	23.561925
D	L100F0	18	48	37.69908
E	L100F0	18	23	18.0641425
A	W0F0	18	20	15.70795
B	W0F0	18	38	29.845105
C	W0F0	18	22	17.278745
D	W0F0	18	34	26.703515
E	W0F0	18	44	34.55749
A	W0F50	18	24	18.84954
B	W0F50	18	65	51.0508375
C	W0F50	18	59	46.3384525
D	W0F50	18	84	65.97339
E	W0F50	18	47	36.9136825
A	W0F100	18	70	54.977825
B	W0F100	18	74	58.119415

Block	Treatment	DAY	Intersects	Length (mm)
D	W0F100	18	65	51.0508375
E	W0F100	18	104	81.68134
A	F10F40	21	84	65.97339
B	F10F40	21	67	52.6216325
C	F10F40	21	37	29.0597075
D	F10F40	21	71	55.7632225
E	F10F40	21	68	53.40703
A	F10F90	21	78	61.261005
B	F10F90	21	94	73.827365
C	F10F90	21	75	58.9048125
D	F10F90	21	62	48.694645
E	F10F90	21	50	39.269875
A	F50F0	21	42	32.986695
B	F50F0	21	28	21.99113
C	F50F0	21	28	21.99113
D	F50F0	21	45	35.3428875
E	F50F0	21	48	37.69908
A	F50F50	21	44	34.55749
B	F50F50	21	44	34.55749
C	F50F50	21	49	38.4844775
D	F50F50	21	94	73.827365
E	F50F50	21	90	70.685775
A	F100F0	21	47	36.9136825
B	F100F0	21	30	23.561925
C	F100F0	21	36	28.27431
D	F100F0	21	54	42.411465
E	F100F0	21	68	53.40703
A	L10F40	21	27	21.2057325
B	L10F40	21	83	65.1879925
C	L10F40	21	109	85.6083275
D	L10F40	21	95	74.6127625
E	L10F40	21	81	63.6171975
A	L10F90	21	78	61.261005
B	L10F90	21	115	90.3207125
C	L10F90	21	108	84.82293
D	L10F90	21	97	76.1835575
E	L10F90	21	100	78.53975
A	L50F0	21	0	0
B	L50F0	21	7	5.4977825
C	L50F0	21	21	16.4933475
D	L50F0	21	67	52.6216325
E	L50F0	21	55	43.1968625
A	L50F50	21	65	51.0508375
B	L50F50	21	103	80.8959425
C	L50F50	21	75	58.9048125
E	L50F50	21	120	94.2477
A	L100F0	21	55	43.1968625
B	L100F0	21	36	28.27431
C	L100F0	21	32	25.13272
D	L100F0	21	57	44.7676575
E	L100F0	21	30	23.561925
A	W0F0	21	46	36.128285
B	W0F0	21	59	46.3384525
C	W0F0	21	30	23.561925
D	W0F0	21	42	32.986695
E	W0F0	21	58	45.553055
A	W0F50	21	32	25.13272
B	W0F50	21	128	100.53088
C	W0F50	21	92	72.25657
D	W0F50	21	91	71.4711725

Block	Treatment	DAY	Intersects	Length (mm)
A	W0F100	21	87	68.3295825
B	W0F100	21	121	95.0330975
C	W0F100	21	136	106.81406
D	W0F100	21	80	62.8318
E	W0F100	21	162	127.234395
A	F10F40	25	102	80.110545
B	F10F40	25	80	62.8318
C	F10F40	25	103	80.8959425
D	F10F40	25	81	63.6171975
E	F10F40	25	105	82.4667375
A	F10F90	25	119	93.4623025
B	F10F90	25	104	81.68134
C	F10F90	25	102	80.110545
D	F10F90	25	82	64.402595
E	F10F90	25	108	84.82293
A	F50F0	25	49	38.4844775
B	F50F0	25	78	61.261005
C	F50F0	25	51	40.0552725
D	F50F0	25	78	61.261005
E	F50F0	25	70	54.977825
A	F50F50	25	91	71.4711725
B	F50F50	25	67	52.6216325
C	F50F50	25	101	79.3251475
D	F50F50	25	142	111.526445
E	F50F50	25	143	112.3118425
A	F100F0	25	90	70.685775
B	F100F0	25	46	36.128285
C	F100F0	25	76	59.69021
D	F100F0	25	114	89.535315
E	F100F0	25	129	101.3162775
A	L10F40	25	46	36.128285
B	L10F40	25	107	84.0375325
C	L10F40	25	182	142.942345
D	L10F40	25	155	121.7366125
E	L10F40	25	119	93.4623025
A	L10F90	25	104	81.68134
B	L10F90	25	151	118.5950225
C	L10F90	25	180	141.37155
D	L10F90	25	187	146.8693325
E	L10F90	25	155	121.7366125
A	L50F0	25	0	0
B	L50F0	25	8	6.28318
C	L50F0	25	34	26.703515
D	L50F0	25	121	95.0330975
E	L50F0	25	74	58.119415
A	L50F50	25	93	73.0419675
C	L50F50	25	119	93.4623025
D	L50F50	25	102	80.110545
E	L50F50	25	170	133.517575
A	L100F0	25	108	84.82293
B	L100F0	25	86	67.544185
C	L100F0	25	52	40.84067
D	L100F0	25	65	51.0508375
E	L100F0	25	43	33.7720925
A	W0F0	25	76	59.69021
B	W0F0	25	80	62.8318
C	W0F0	25	60	47.12385
D	W0F0	25	69	54.1924275
E	W0F0	25	65	51.0508375
A	W0F50	25	65	51.0508375
B	W0F50	25	149	117.0242275
C	W0F50	25	107	84.0375325

Block	Treatment	DAY	Intersects	Length (mm)
E	W0F50	25	98	76.968955
A	W0F100	25	104	81.68134
B	W0F100	25	192	150.79632
C	W0F100	25	178	139.800755
D	W0F100	25	139	109.1702525
E	W0F100	25	192	150.79632
A	F10F40	28	140	109.95565
B	F10F40	28	110	86.393725
C	F10F40	28	171	134.3029725
D	F10F40	28	116	91.10611
E	F10F40	28	147	115.4534325
A	F10F90	28	184	144.51314
B	F10F90	28	118	92.676905
C	F10F90	28	140	109.95565
D	F10F90	28	123	96.6038925
E	F10F90	28	159	124.8782025
A	F50F0	28	78	61.261005
B	F50F0	28	154	120.951215
C	F50F0	28	96	75.39816
D	F50F0	28	124	97.38929
E	F50F0	28	124	97.38929
A	F50F50	28	149	117.0242275
B	F50F50	28	71	55.7632225
C	F50F50	28	134	105.243265
D	F50F50	28	145	113.8826375
E	F50F50	28	161	126.4489975
A	F100F0	28	162	127.234395
B	F100F0	28	73	57.3340175
C	F100F0	28	126	98.960085
D	F100F0	28	174	136.659165
E	F100F0	28	169	132.7321775
A	L10F40	28	70	54.977825
B	L10F40	28	168	131.94678
C	L10F40	28	260	204.20335
D	L10F40	28	212	166.50427
E	L10F40	28	187	146.8693325
A	L10F90	28	155	121.7366125
B	L10F90	28	214	168.075065
C	L10F90	28	240	188.4954
D	L10F90	28	226	177.499835
E	L10F90	28	256	201.06176
A	L50F0	28	6	4.712385
B	L50F0	28	25	19.6349375
C	L50F0	28	62	48.694645
D	L50F0	28	174	136.659165
A	L50F50	28	121	95.0330975
B	L50F50	28	208	163.36268
C	L50F50	28	283	222.2674925
D	L50F50	28	229	179.8560275
E	L50F50	28	219	172.0020525
A	L100F0	28	158	124.092805
B	L100F0	28	190	149.225525
C	L100F0	28	84	65.97339
D	L100F0	28	81	63.6171975
E	L100F0	28	115	90.3207125
A	W0F0	28	110	86.393725
B	W0F0	28	86	67.544185
C	W0F0	28	116	91.10611
D	W0F0	28	115	90.3207125
E	W0F0	28	101	79.3251475
A	W0F50	28	103	80.8959425
B	W0F50	28	180	141.37155

Block	Treatment	DAY	Intersects	Length (mm)
D	W0F50	28	234	183.783015
E	W0F50	28	156	122.52201
A	W0F100	28	244	191.63699
B	W0F100	28	226	177.499835
C	W0F100	28	229	179.8560275
D	W0F100	28	170	133.517575
E	W0F100	28	248	194.77858
A	F10F40	32	216	169.64586
B	F10F40	32	196	153.93791
C	F10F40	32	341	267.8205475
D	F10F40	32	170	133.517575
E	F10F40	32	170	133.517575
A	F10F90	32	186	146.083935
B	F10F90	32	300	235.61925
C	F10F90	32	278	218.340505
D	F10F90	32	256	201.06176
E	F10F90	32	196	153.93791
A	F50F0	32	215	168.8604625
B	F50F0	32	306	240.331635
C	F50F0	32	162	127.234395
D	F50F0	32	153	120.1658175
E	F50F0	32	145	113.8826375
A	F50F50	32	199	156.2941025
B	F50F50	32	167	131.1613825
C	F50F50	32	221	173.5728475
D	F50F50	32	261	204.9887475
E	F50F50	32	238	186.924605
A	F100F0	32	413	324.3691675
B	F100F0	32	146	114.668035
C	F100F0	32	235	184.5684125
D	F100F0	32	336	263.89356
E	F100F0	32	309	242.6878275
A	L10F40	32	147	115.4534325
B	L10F40	32	334	262.322765
C	L10F40	32	354	278.030715
D	L10F40	32	278	218.340505
E	L10F40	32	220	172.78745
A	L10F90	32	405	318.0859875
B	L10F90	32	427	335.3647325
C	L10F90	32	346	271.747535
D	L10F90	32	353	277.2453175
E	L10F90	32	477	374.6346075
A	L50F0	32	78	61.261005
B	L50F0	32	115	90.3207125
D	L50F0	32	262	205.774145
E	L50F0	32	204	160.22109
A	L50F50	32	269	211.2719275
B	L50F50	32	380	298.45105
C	L50F50	32	455	357.3558625
D	L50F50	32	437	343.2187075
E	L50F50	32	326	256.039585
A	L100F0	32	296	232.47766
B	L100F0	32	305	239.5462375
C	L100F0	32	151	118.5950225
D	L100F0	32	134	105.243265
E	L100F0	32	299	234.8338525
A	W0F0	32	148	116.23883
B	W0F0	32	168	131.94678
C	W0F0	32	142	111.526445
D	W0F0	32	180	141.37155
E	W0F0	32	127	99.7454825
A	W0F50	32	204	160.22109

Block	Treatment	DAY	Intersects	Length (mm)
C	W0F50	32	253	198.7055675
D	W0F50	32	318	249.756405
E	W0F50	32	285	223.8382875
A	W0F100	32	467	366.7806325
B	W0F100	32	407	319.6567825
C	W0F100	32	342	268.605945
D	W0F100	32	225	176.7144375
E	W0F100	32	289	226.9798775
A	F10F40	35	423	332.2231425
B	F10F40	35	437	343.2187075
C	F10F40	35	563	442.1787925
D	F10F40	35	365	286.6700875
E	F10F40	35	317	248.9710075
A	F10F90	35	400	314.159
B	F10F90	35	545	428.0416375
C	F10F90	35	549	431.1832275
D	F10F90	35	526	413.119085
E	F10F90	35	354	278.030715
A	F50F0	35	440	345.5749
B	F50F0	35	588	461.81373
C	F50F0	35	312	245.04402
D	F50F0	35	270	212.057325
E	F50F0	35	246	193.207785
A	F50F50	35	406	318.871385
B	F50F50	35	298	234.048455
C	F50F50	35	379	297.6656525
D	F50F50	35	504	395.84034
E	F50F50	35	528	414.68988
A	F100F0	35	651	511.2937725
B	F100F0	35	439	344.7895025
C	F100F0	35	441	346.3602975
D	F100F0	35	524	411.54829
E	F100F0	35	652	512.07917
A	L10F40	35	322	252.897995
B	L10F40	35	627	492.4442325
A	L10F90	35	622	488.517245
C	L10F90	35	610	479.092475
D	L10F90	35	701	550.5636475
E	L10F90	35	962	755.552395
A	L50F50	35	586	460.242935
B	L50F50	35	663	520.7185425
C	L50F50	35	700	549.77825
D	L50F50	35	559	439.0372025
E	L50F50	35	576	452.38896
A	L100F0	35	632	496.37122
B	L100F0	35	405	318.0859875
C	L100F0	35	228	179.07063
D	L100F0	35	312	245.04402
E	L100F0	35	647	508.1521825
A	W0F100	35	793	622.8202175
B	W0F100	35	630	494.800425
C	W0F100	35	610	479.092475
D	W0F100	35	500	392.69875
E	W0F100	35	565	443.7495875
A	F10F90	39	809	635.3865775
B	F10F90	39	726	570.198585
C	F10F90	39	813	638.5281675
D	F10F90	39	733	575.6963675
E	F10F90	39	575	451.6035625
A	F50F50	39	805	632.2449875
B	F50F50	39	500	392.69875
C	F50F50	39	596	468.09691

Block	Treatment	DAY	Intersects	Length (mm)
E	F50F50	39	838	658.163105
A	F100F0	39	988	775.97273
B	F100F0	39	818	642.455155
C	F100F0	39	639	501.8690025
D	F100F0	39	839	658.9485025
E	F100F0	39	1064	835.66294
A	L10F90	39	844	662.87549
B	L10F90	39	913	717.0679175
C	L10F90	39	937	735.9174575
D	L10F90	39	915	718.6387125
E	L10F90	39	1182	928.339845
A	L50F50	39	945	742.2006375
B	L50F50	39	927	728.0634825
D	L50F50	39	716	562.34461
E	L50F50	39	629	494.0150275
A	L100F0	39	995	781.4705125
B	L100F0	39	599	470.4531025
C	L100F0	39	560	439.8226
D	L100F0	39	655	514.4353625
E	L100F0	39	1060	832.52135
A	W0F100	39	1000	785.3975
B	W0F100	39	985	773.6165375
C	W0F100	39	955	750.0546125
D	W0F100	39	813	638.5281675
E	W0F100	39	935	734.3466625

Appendix 4.3: Nutrient content in plant tissue.

Treatment	Block	% Ca	% P	% Mg	% K	% Na	PPM Fe	PPM Zn	PPM Cu	PPM Mn	PPM Mo	% S
F100F0	1	1.84	0.65	0.45	3.85	0.176	44	47	4	12	6	0.82
F100F0	2	2.06	0.75	0.48	4.43	0.174	84	50	6	15	6.5	0.87
F100F0	3	1.7	0.68	0.41	4.33	0.168	35	44	5	13	5.2	0.82
F100F0	4	1.94	0.79	0.46	4.54	0.197	73	54	6	16	6.5	0.97
F100F0	5	1.7	0.67	0.41	3.94	0.167	42	50	4	16	5.2	0.76
F10F40	1	1.89	1.35	0.43	4.95	0.12	43	50	5	10	9.5	0.99
F10F40	2	1.93	1.08	0.43	4.55	0.136	46	44	5	9	8	0.93
F10F40	3	1.73	1.14	0.39	4.61	0.138	38	45	6	9	7	0.83
F10F40	4	1.87	1.47	0.42	4.97	0.121	39	52	5	11	6.9	0.93
F10F40	5	1.61	1.17	0.37	4.79	0.111	37	47	6	11	6.6	0.91
F10F90	1	1.76	1.03	0.42	4.41	0.144	43	44	7	12	5.9	0.96
F10F90	2	1.76	0.9	0.43	4.39	0.162	58	45	5	12	5.5	0.9
F10F90	3	1.59	0.9	0.4	4.84	0.192	45	43	6	12	6	0.78
F10F90	4	1.49	0.78	0.38	4.08	0.172	42	49	5	10	4.8	0.69
F10F90	5	1.78	1.07	0.42	5.54	0.141	52	55	5	15	5.8	0.93
F50F0	1	1.82	1.11	0.41	4.81	0.138	35	57	5	12	8.6	0.96
F50F0	2	1.73	0.93	0.39	4.38	0.105	56	52	5	11	6.3	0.9
F50F0	3	1.4	0.84	0.32	4.05	0.132	73	46	3	10	5.1	0.65
F50F0	4	1.58	0.87	0.35	4.66	0.126	94	48	11	13	5.7	0.81
F50F0	5	1.49	0.87	0.34	4.26	0.138	50	51	5	11	6.2	0.81
F50F50	1	1.85	0.9	0.43	5.28	0.205	49	55	8	13	5.5	0.85
F50F50	2	1.56	0.81	0.39	4.77	0.201	41	37	6	10	5.2	0.79
F50F50	3	1.6	0.86	0.4	4.64	0.194	44	42	5	12	5.7	0.77
F50F50	4	1.8	0.83	0.43	4.61	0.183	48	43	5	11	5.3	0.82
F50F50	5	1.93	0.86	0.46	4.51	0.2	77	47	7	12	7.2	0.98
L100F0	1	1.67	0.75	0.37	5.33	0.326	281	50	8	32	10.8	0.75
L100F0	2	1.51	0.79	0.35	4.82	0.359	49	44	5	27	7.7	0.74
L100F0	3	1.51	0.83	0.35	5.48	0.394	49	51	3	32	7.6	0.72
L100F0	4	1.61	0.78	0.38	5.71	0.523	83	47	4	29	6.1	0.7
L100F0	5	1.68	0.94	0.39	6.48	0.446	49	52	5	28	6.9	0.8
L10F40	1	2.05	1.15	0.48	5.21	0.186	40	44	5	10	7.4	0.91
L10F40	2	2.36	1.18	0.55	5.2	0.165	64	65	6	10	9.9	1.31
L10F40	3	1.98	0.95	0.44	4.41	0.123	33	43	6	10	8.1	0.89
L10F40	4	1.56	0.91	0.38	4.18	0.147	29	41	4	9	6.2	0.85
L10F40	5	1.79	1.06	0.45	4.57	0.116	94	53	6	11	8.4	0.95
L10F90	1	1.82	0.87	0.42	5.08	0.196	47	44	7	12	6.9	0.86
L10F90	2	1.73	0.85	0.41	4.48	0.145	42	36	5	11	6.1	0.81
L10F90	3	1.74	0.91	0.42	4.89	0.172	39	39	5	10	5.7	0.8
L10F90	4	1.74	0.89	0.41	4.37	0.176	59	41	6	10	5.9	0.81
L10F90	5	1.43	0.7	0.35	4.03	0.157	33	35	4	9	4.9	0.71
L50F0	1	2.09	0.89	0.49	8.88	0.518	77	58	5	31	5.6	0.92
L50F0	2	1.95	1.29	0.45	6.56	0.278	123	63	16	27	8.9	0.95
L50F0	3	1.89	1.08	0.43	6.22	0.312	44	55	5	24	8.6	0.91
L50F0	4	1.68	0.87	0.39	4.5	0.233	113	51	5	21	9.7	0.81
L50F0	5	1.79	1.17	0.39	5.54	0.28	42	56	4	26	9.9	0.88
L50F50	1	1.76	0.97	0.4	5.93	0.276	82	45	7	19	8.5	0.95
L50F50	2	1.68	0.81	0.38	4.79	0.232	38	40	6	14	8.2	0.8
L50F50	3	1.59	0.82	0.38	4.72	0.221	38	42	4	16	7.9	0.74
L50F50	4	1.67	0.9	0.4	4.92	0.262	44	46	6	18	8.5	0.77
L50F50	5	1.53	0.86	0.37	5.29	0.3	40	44	6	18	7.5	0.84
W0F0	1	2.69	1.55	0.57	4.99	0.141	188	69	22	13	10.9	1.4
W0F0	2	2.74	1.79	0.59	5.19	0.135	40	65	5	12	10.2	1.41
W0F0	3	2.22	1.58	0.52	5.05	0.167	30	60	6	11	8.6	1.32
W0F0	4	2.34	1.56	0.52	4.49	0.154	38	61	4	10	9.2	1.27
W0F0	5	2	1.34	0.45	4.69	0.127	31	55	5	0	7.9	1
W0F100	2	1.43	0.79	0.36	4	0.154	31	35	5	8	4.6	0.62
Treatment	**Block**	**% Ca**	**% P**	**% Mg**	**% K**	**% Na**	**PPM Fe**	**PPM Zn**	**PPM Cu**	**PPM Mn**	**PPM Mo**	**% S**

W0F100	3	1.68	0.88	0.41	3.97	0.145	59	37	7	8	5.9	0.77
W0F100	4	1.61	0.85	0.39	4.13	0.129	37	34	6	9	4.7	0.73
W0F100	5	1.71	0.95	0.41	4.34	0.156	41	35	6	10	5.6	0.78
W0F50	1	2.57	1.47	0.56	5.14	0.138	37	53	0	11	9.8	1.17
W0F50	2	2.17	1.22	0.49	4.62	0.147	39	46	5	9	8.4	1.08
W0F50	3	2.03	1.13	0.47	4.62	0.159	35	44	5	8	8.8	1.08
W0F50	4	1.85	1.16	0.44	4.35	0.105	28	41	4	9	6.7	0.93
W0F50	5	1.79	1.22	0.41	4.55	0.126	28	41	5	10	7.2	1.02

Appendix 4.4: Electrical conductivity (EC) data.

Blk	Treatment	EC(1:2) - mS/cm	ECe conversion
1	F100F0	0.698	2.27713568
2	F100F0	0.844	2.61120704
3	F100F0	0.821	2.55857936
4	F100F0	1.266	3.57681056
5	F100F0	1.452	4.00240832
1	F10F90	0.706	2.29544096
2	F10F90	0.783	2.47162928
3	F10F90	1.096	3.18782336
4	F10F90	0.628	2.11696448
5	F10F90	0.748	2.39154368
1	F50F50	0.735	2.3617976
2	F50F50	0.828	2.57459648
3	F50F50	0.909	2.75993744
4	F50F50	1.096	3.18782336
5	F50F50	0.885	2.7050216
1	L100F0	0.936	2.82171776
2	L100F0	1.472	4.04817152
3	L100F0	2.421	6.21963536
4	L100F0	1.468	4.03901888
5	L100F0	1.963	5.17165808
2	L10F90	0.654	2.17645664
3	L10F90	0.577	2.00026832
4	L10F90	0.931	2.81027696
5	L10F90	1.397	3.87655952
1	L50F50	0.819	2.55400304
2	L50F50	0.836	2.59290176
3	L50F50	1.045	3.0711272
4	L50F50	1.153	3.31824848
5	L50F50	1.587	4.31130992
1	W0F100	0.68	2.2359488
2	W0F100	0.748	2.39154368
3	W0F100	0.879	2.69129264
4	W0F100	0.817	2.54942672
5	W0F100	1.747	4.67741552

Appendix 4.5: SPAD readings.

Blk	Treatment	SPAD1	SPAD2	SPAD3	SPAD4	SPAD5	AVG SPAD
1	W0F0	12.6	18.7	12.9	12.3	12.2	13.74
1	W0F50	23.7	24.1	23.5	21.4	21.7	22.88
1	W0F100	23.8	21.2	24.8	26	25.1	24.18
1	L10F40	17.5	21.4	23.1	20.9	19.9	20.56
1	L10F90	27	24.3	22.5	25.6	24.3	24.74
1	L50F0	18.2	19.5	19.9	19.4	20	19.4
1	L50F50	19.6	21.9	27.8	23.1	24.9	23.46
1	L100F0	24.7	25.8	24.8	25.9	21.7	24.58
1	F10F40	20.9	20.7	17.8	24.6	21.7	21.14
1	F10F90	24.2	29.7	28.5	26.9	24.4	26.74
1	F50F0	25.5	25.3	24.8	24.5	22.6	24.54
1	F50F50	23.5	23.5	22.3	27.9	25.5	24.54
1	F100F0	21.7	20.3	22.2	20.2	20	20.88
2	W0F0	13.2	12.1	11	14.7	15.6	13.32
2	W0F50	22.9	22.7	22.4	20.5	22.6	22.22
2	W0F100	23.1	22.5	22.6	22.9	20	22.22
2	L10F40	21.7	19	22.5	17.2	18.4	19.76
2	L10F90	27	28.4	27.5	23.4	22.2	25.7
2	L50F0	21.2	20.5	21.5	21.3	17.9	20.48
2	L50F50	26.5	29.9	27.2	22.8	22.3	25.74
2	L100F0	25.3	26.6	24.1	23	25	24.8
2	F10F40	20.7	23.3	25.4	25.4	20.9	23.14
2	F10F90	22	24.6	29.9	27.1	21.4	25
2	F50F0	24.7	22.8	23.2	24.2	22.3	23.44
2	F50F50	25.3	27.5	26.6	24.1	22.4	25.18
2	F100F0	23.6	22.4	23.9	25	23.2	23.62
3	W0F0	14.5	14.7	14.3	11.1	13.8	13.68
3	W0F50	22.1	20.4	20.5	20.5	18.4	20.38
3	W0F100	23.2	28.4	25.4	26.6	21.1	24.94
3	L10F40	22.2	23.7	24.4	22.3	20.2	22.56
3	L10F90	22.6	21.2	26.8	25	23.8	23.88
3	L50F0	19.2	20	20.3	18.1	19.7	19.46
3	L50F50	22.9	24.7	26.3	24.2	21.9	24
3	L100F0	24.7	24.1	26.3	21.3	21.3	23.54
3	F10F40	18.6	22.1	22.9	20	21.8	21.08
3	F10F90	24	24.5	26.8	27.3	26.5	25.82
3	F50F0	24.9	23.1	24.3	22.1	25.3	23.94
3	F50F50	22.9	21.8	25.9	21.3	23.4	23.06
3	F100F0	26.3	25.4	26.8	22.3	22	24.56
4	W0F0	12.7	10.1	7.1	9.3	9.7	9.78
4	W0F50	20.4	19.8	19.1	19.9	17.4	19.32
4	W0F100	25.4	19.8	26.3	24	22.9	23.68
4	L10F40	21.8	21.5	23.4	20	20.9	21.52
4	L10F90	24	22.2	23.4	24.2	21.2	23
4	L50F0	26.3	28.7	26.6	26.4	25.5	26.7
4	L50F50	24.1	27.4	23.5	22.1	22.2	23.86
4	L100F0	23.9	22.8	22.3	21.4	21.1	22.3
4	F10F40	24.2	24.1	25.6	22.9	21.9	23.74
4	F10F90	23.8	24.7	25.9	22.1	22.4	23.78
4	F50F0	25.8	23.3	24.2	27.2	25.7	25.24
4	F50F50	25.1	25.3	27.5	26.6	25.5	26
4	F100F0	24.9	29	27.9	24.3	27.6	26.74
5	W0F0	13.3	14.5	14.9	12	14.5	13.84
5	W0F50	25.5	22.2	21.6	19.7	22.4	22.28
5	W0F100	25.1	24.3	29	24.7	22.2	25.06
5	L10F40	22.4	22.6	23.2	21.2	18.7	21.62
5	L10F90	25.6	27	28.9	28.8	24.9	27.04
5	L50F0	22.6	25.4	25.7	22.4	21.7	23.56
5	L50F50	26	27.9	28.7	28.7	27.1	27.68

Blk	Treatment	SPAD1	SPAD2	SPAD3	SPAD4	SPAD5	AVG SPAD
5	L100F0	23.7	24.3	26.4	25.1	24.3	24.76
5	F10F40	22.2	23.6	25.5	25.8	24.5	24.32
5	F10F90	23.7	29.5	26.1	21.8	20.5	24.32
5	F50F0	28	24.1	26.9	26.5	27.3	26.56
5	F50F50	24.7	28.9	26.1	23.4	21.3	24.88
5	F100F0	26.4	26.8	25.6	26.9	26.3	26.4

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